





© 2003 raytest Isotopenmeßgeräte GmbH, Benzstr. 4, D-75334 Straubenhardt, Germany.

All rights reserved. This product or document is protected by copyright and distributed under licenses restricting its use, copying, distribution and decompilation. No part of this product or document may be reproduced in any form by any means without prior written authorization of raytest and its licensors, if any.

RESTRICTED RIGHTS LEGEND: Use, duplication, or disclosure by the government is subject to restrictions as set forth in subparagraph (c)(1)(ii) of the Rights in Technical Data and Computer Software clause at DFARS 252.227-7013 and FAR 52.227-19.

The product described in this manual may be protected by one or more U.S. patents, foreign patents, or pending applications.

TRADEMARKS

AIDA, AIDA Image Analyzer, AIDA Annotation, AIDA AIDA Array Metrix, AIDA Array Compare, AIDA Multi Labeling, AIDA 1D Evaluation, AIDA 1D Densitometry, AIDA 2D Densitometry, AIDA Easy Array, AIDA Whole Body Autoradiography, AIDA 1D Thin Layer Chromatography and AIDA 2D Thin Layer Chromatography are trademarks or registered trademarks of raytest in the United States and may be protected as trademark in other countries.

All other product, service, or company names mentioned herein are claimed as trademarks and trade names by their respective companies.

THIS PUBLICATION IS PROVIDED "AS IS" WITHOUT WARRANTY OF ANY KIND, EITHER EXPRESS OR IMPLIED, INCLUDING, BUT NOT LIMITED TO, THE IMPLIED WARRANTIES OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, OR NON-INFRINGEMENT.

THIS PUBLICATION COULD INCLUDE TECHNICAL INACCURACIES OR TYPOGRAPHICAL ERRORS. CHANGES ARE PERIODICALLY ADDED TO THE INFORMATION HEREIN, THESE CHANGES WILL BE INCORPORATED IN NEW EDITIONS OF THE PUBLICATION.

raytest MAY MAKE IMPROVEMENTS AND/OR CHANGES IN THE PRODUCT(S) AND/OR THE PROGRAMS(S) DESCRIBED IN THIS PUBLICATION AT ANY TIME.

Contents



1	Introduction	5
	Overview	5
	Starting 2D Densitometry	6
2	Using 2D Densitometry	7
	Data Windows in 2D Densitometry	7
	Image Window in 2D Densitometry	7
	Contextual Menu of the Image Window	8
	Image Overlays	9
	Region Report Window	11
	Data Description	11
	Contextual Menu of the Region Report Window	15
	Region Calibration Window	22
	Graphics Pane of the Regions Calibration Window	23
	Contextual Menu of the Region Calibration Window	25
	Additional Toolboxes in 2D Densitometry	29
	Region Determination Toolbox	29
	Region Creation Pane	31
	Integration Pane	33
	Report Pane	35
	Region Label and Name Pane	36
	Actual Region Pane	42
	Region Calibration Toolbox	43
	Method Pane	44
	Standard Pane	44
	Calibration Curve Pane	46
	Publishing Layout Toolbox in 2D Densitometry	48
	Print Options for Region Report	49
	Print Options for Region Calibration	49
	Print	50
	Preview	51



Export and Import	53
Exporting Templates	53
Exporting Region Report	54
Exporting Region Calibration	56
Automatic Export of the Region Reports/Region Calibrations	59
Importing Region Calibration	63
3 Performing 2D Evaluations	65
Assigning Regions	65
Using Arrays (Matrices).	71
Defining a Background Signal.	74
Performing Calibrations.	75
Using Nametables	79
Creating and Editing Work Nametables	79
Assigning Names to Regions	82



Overview

Some methods result in digital images, where the signal intensity of certain areas of these images need to be evaluated (e.g. 2D protein gels, dot blot assays, images of fluorescent multiwell plates, autoradiographs of tissue cuts, in situ hybridizations, etc.). To enable quantification, these areas must be marked by an enclosure of the appropriate shape (rectangle, circle, free drawn object).

The AIDA 2D Densitometry module is used to evaluate the intensity within two-dimensional shapes (called regions).

The signal intensity can be integrated (Quantity) over the entire region. For concentration calibration, the intensity is assigned to the corresponding area (of the region). It is important to enclose the area of interest (e.g. the spot) entirely with the region when using the integration option.

The integrated signal intensity can be compared between regions (relative comparison) or normalized by standards in the same image, or even in other samples of the same series.

However, it is very important to understand and acknowledge the limits of these evaluations to prevent pitfalls that will lead to results of very limited value. Thus some suggestions for practical considerations for the sample preparation and image capturing will be given at the various evaluation section.

It is important that all comparisons of samples to standards should be validated.

Evaluations get more accurate with more standard values, which should be well scattered around the intensities to be examined. When in doubt it might be a good idea to check the reproducibility of 'known' results. Including and evaluating multiple dilutions of samples will also make quantitative estimations safer.



1

Starting 2D Densitometry

To select the **2D Densitometry** mode:

- 1 Click the **Evaluation** button in the toolbar.

A dialog box appears offering you a list of available evaluation modes.

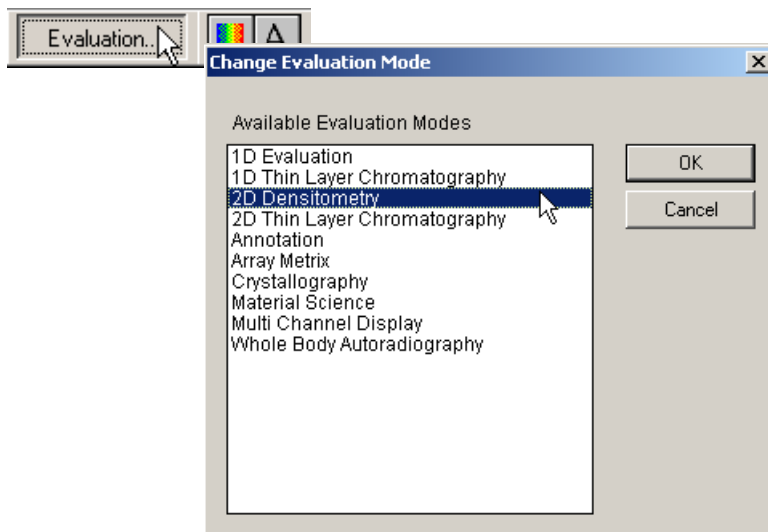


Figure 1-1 Changing Evaluation Mode

The list entries reflect the installed AIDA application modules.

- 2 Select the **2D Densitometry** list item and click **OK**.

Note – This evaluation mode is only available if you purchased the AIDA 2D Densitometry module.



Data Windows in 2D Densitometry

In addition to the AIDA standard data windows **Image** and **Histogram** the following data windows are available in the **2D Densitometry** mode:

- Region Report
- Region Calibration

You can access these data windows by choosing the appropriate entry (**2D Region Report**, **Region Calibration** respectively) from the **View** menu.

Image Window in 2D Densitometry

All data are evaluated from the **Image** window. Although it might not always be on top, it is always open. The title bar of the **Image** window shows the full file path of the image.

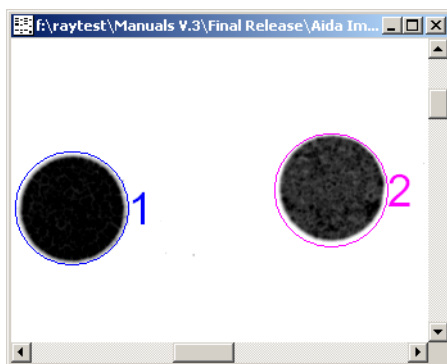


Figure 2-1 Image Window Example

You can copy the content of the **Image** window to the clipboard using the **Copy Content** command of the **Edit** menu. See the “Export Image” section in the “AIDA Image Analyzer Basic Concepts and Features User's Manual” for further explanation.

Contextual Menu of the Image Window

In 2D Densitometry, the contextual menu of the **Image** window looks slightly different depending on the type of overlay, you have chosen:

Single Selection:

If you have selected a single overlay (region) only, the contextual menu, that opens after right-clicking the respective object, provides you with the possible commands for this specific selection mode in addition to the standard editing functions like **Cut**, **Copy**, **Paste**, **Delete** and **Duplicate**. These include commands for labeling regions and setting integration limits.

Multiple Selection:

If you have selected multiple overlays (regions), the resulting contextual menu is considerably reduced (compared with the single selection contextual menu), showing only those commands available in this selection mode. The provided menu items form a subset of the functions available in the single selection mode.

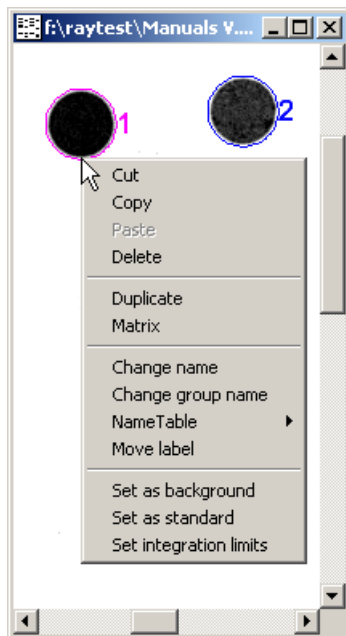


Figure 2-2 Contextual Menu (Single Object Selected)

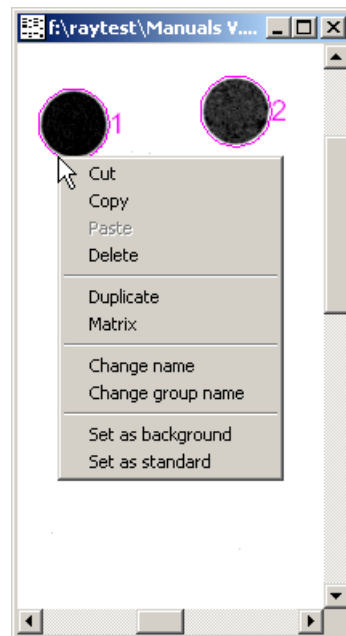


Figure 2-3 Contextual Menu (Multiple Objects Selected)

If no region is selected, only the basic editing functions of the standard contextual menu described in the “AIDA Image Analyzer Basic Concepts and Features User's Manual” will be accessible.

Note – All of the functions in these contextual menus are also accessible through different means (menus, buttons, toolboxes, windows). They will be explained in the respective sections of this manual. However, it is often more convenient to activate the function through the readily accessible background menu. This will make repetitive and routine tasks faster and more convenient.

Image Overlays

You can specify the label type and font you want to apply to the overlays (lanes and bands) using the **Image Overlays** command of the **Options** menu.

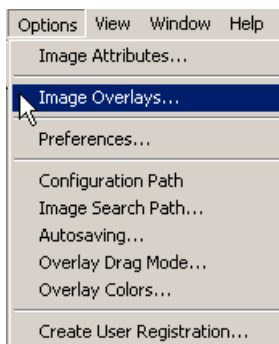


Figure 2-4 Options Menu: Opening the Image Overlays Dialog Box

After choosing **Image Overlays** from the **Options** menu, the **Image Overlays** dialog box opens allowing you to specify the type (**None**, **Number**, **Name**, **Free Choice**), font, font size, and font style (bold, italic, roman typeface on white background) for labeling. For details, see the appropriate sections below.

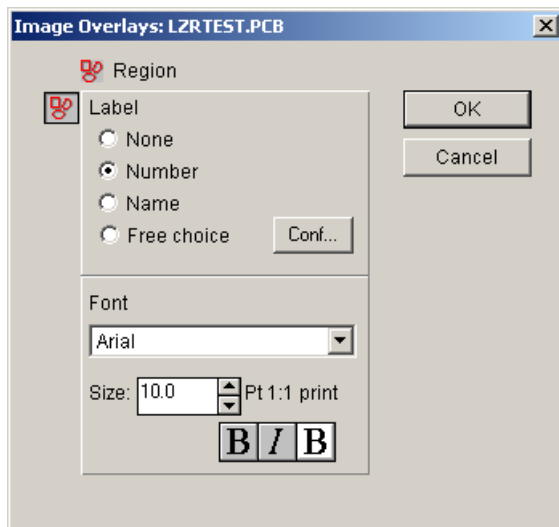
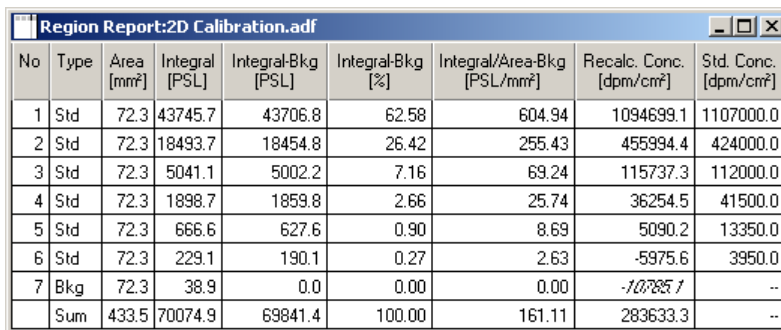


Figure 2-5 Image Overlays Dialog Box

Region Report Window

All information about the evaluation of the assigned regions can be found and configured to the specific needs in the **Region Report**.

The **Region Report** window is shown by clicking this button  in the **Region Determination** toolbox or by choosing **2D Region Report** from the **View** menu.



No	Type	Area [mm²]	Integral [PSL]	Integral-Bkg [PSL]	Integral-Bkg [%]	Integral/Area-Bkg [PSL/mm²]	Recalc. Conc. [dpm/cm²]	Std. Conc. [dpm/cm²]
1	Std	72.3	43745.7	43706.8	62.58	604.94	1094699.1	1107000.0
2	Std	72.3	18493.7	18454.8	26.42	255.43	455994.4	424000.0
3	Std	72.3	5041.1	5002.2	7.16	69.24	115737.3	112000.0
4	Std	72.3	1898.7	1859.8	2.66	25.74	36254.5	41500.0
5	Std	72.3	666.6	627.6	0.90	8.69	5090.2	13350.0
6	Std	72.3	229.1	190.1	0.27	2.63	-5975.6	3950.0
7	Bkg	72.3	38.9	0.0	0.00	0.00	-10785.1	--
	Sum	433.5	70074.9	69841.4	100.00	161.11	283633.3	--

Figure 2-6 Region Report Window

Data Description

Available **Columns** in the **Region Report**:

Table 2-1 Columns Available in the Region Report Window

Column	Short Description
No	The number of the region.
Grp	The group number to which the region belongs (0 = no group selected for this region).
Name	The name of the region as it was assigned by the user.
Grp Name	The name of the group assigned by the user to which the region belongs.
Type	The type of the region (e. g. bkg – background)
Int. Limits	Integration limits for the region.
Position [<i>length unit</i>]	The position of the region as determined by x and y coordinates in the image.

signal unit and *length unit* = units of measurement, depending on the type of the imaging device used to obtain the image and the options set in the **Image Attributes** dialog box.

concentration unit and *quantity unit* = unit of measurement, specified by the user through calibration.

Table 2-1 Columns Available in the Region Report Window

Column	Short Description
Position X [<i>length unit</i>]	The position of the region as determined by x coordinate in the image
Position Y [<i>length unit</i>]	The position of the region as determined by y coordinate in the image
Area [<i>length unit</i> ²]	The calculated area of the region .
Area [%]	The percentage of the region area compared to the reference row in the group of the region (e.g. the sum of all evaluated areas in a group).
Integral [<i>signal unit</i>]	The calculated intensity of the region .
Integral [%]	The percentage of the intensity of the region compared to the reference row in the group of the region.
Integral – Bkg [<i>signal unit</i>]	The background intensity is subtracted from the calculated intensity of the region to result in the signal intensity of the region. For each group of regions the background is determined in the following order: the local background area around the region, or the local background defined for the group, or the general background defined in group 0, whatever is available.
Integral – Bkg [%]	The percentage of the background corrected intensity of the region compared to the reference row in the group of the region.
Integral / Area [<i>signal unit</i> / <i>length unit</i> ²]	The intensity per area.
Integral / Area [%]	The percentage of the intensity per pixel of the region compared to the reference row in the group of the region.
Integral / Area – Bkg [<i>signal unit</i> / <i>length unit</i> ²]	The background intensity is subtracted from the calculated intensity of the region and this signal intensity is divided by the area.
Integral / Area – Bkg [%]	The percentage of the background-corrected intensity per pixel of the region compared to the reference row in the group of the region.

signal unit and *length unit* = units of measurement, depending on the type of the imaging device used to obtain the image and the options set in the **Image Attributes** dialog box.

concentration unit and *quantity unit* = unit of measurement, specified by the user through calibration.

Table 2-1 Columns Available in the Region Report Window

Column	Short Description
Std. Quantity [<i>quantity unit</i>]	The quantity of the region, as entered for the calculation of the standard curve. With the Quantity Standards the background corrected Intensity values of the regions are calibrated.
Std. Quantity [%]	The percentage of the quantity standard value compared to the reference row in the group of the region.
Recalc. Quantity [<i>quantity unit</i>]	A calculation of the quantity in the region, based on the current calibration curve.
Recalc. Quantity [%]	The percentage of the quantity for the region (determined on the basis of the calibration curve) compared to the reference row in the group of the region.
Std. Conc. [<i>concentration unit</i>]	The concentration of the region, as entered for the calculation of the standard curve. With the Concentration Standards the background corrected Intensity per Area values of the regions are calibrated.
Std. Conc. [%]	The percentage of the concentration standard value compared to the reference row in the group of the region.
Recalc. Conc. [<i>concentration unit</i>]	A calculation of the concentration in the region, based on the current calibration curve.
Recalc. Conc. [%]	The percentage of the quantity for the region (determined on the basis of the calibration curve) compared to the reference row in the group of the region .

signal unit and *length unit* = units of measurement, depending on the type of the imaging device used to obtain the image and the options set in the **Image Attributes** dialog box.

concentration unit and *quantity unit* = unit of measurement, specified by the user through calibration.

The following additional rows can be found or created exclusively in the **Region Report**:

Table 2-2 Additional Rows in the Region Report

Sum	Displays the scientifically correct sum for the selected column (e.g. $\Sigma (\text{Intensity} / \text{Area})$, means $\Sigma \text{ Intensities} / \Sigma \text{ Areas}$). Different groups get individual sums.
Ttl (Total)	Displays the values of the selected column of Ttl region in the group. For group 0 the Ttl region is the entire image.
Remainder	Ttl – Sum

Quantity results in *italics* indicate that they lay outside of the calibration curve values and therefore are prone to significant error.

You can select the number and type of columns you want to display using the contextual menu of any column in the window (see below).

To change the column order:

- 1 Click the heading of the column to be moved.
- 2 Press the mouse button and drag to the desired position in the table.
- 3 Release the mouse button.

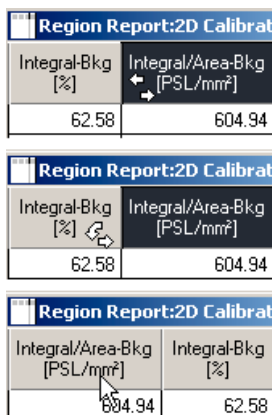


Figure 2-7 Changing Column Order

To change the column width:

- 1 Position the mouse pointer over the border of the column whose width you want to change.
- 2 Click and drag the border in the appropriate direction.
- 3 Release the mouse button:

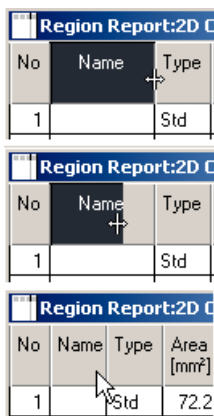


Figure 2-8 Changing Column Width

Contextual Menu of the Region Report Window

Each column in the **Result Table** has a contextual menu attached. To open the contextual menu, right-click on the column heading (gray area).

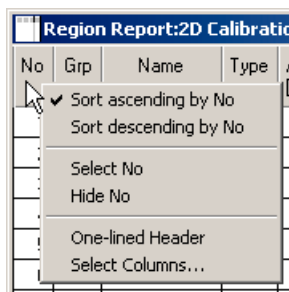


Figure 2-9 Contextual Menu of the Region Report Window

With the first two commands of the contextual menu, you can sort the table of the **Region Report** by any value column in ascending order (A to Z or zero to 9) or descending order (Z to A or 9 to zero). Just choose the appropriate menu item (**Sort Ascending by/Sort Descending by**).

The **Select/Unselect** commands of the contextual menu are used to select/deselect a column to export. The column heading of selected columns are highlighted.

To hide a column, select the **Hide** command from its contextual menu. You can recover hidden columns using the **Select Columns** command (see below).

To specify the decimal digits for the value output in a column, choose **Settings of** from the contextual menu and enter the value for the decimal digits in the dialog box displayed.

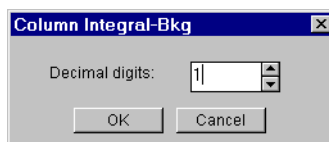


Figure 2-10 Setting Output Format for Columns

Use the **One-lined Header/Two-lined Header** command to reduce or expand the column heading by one line (the second line indicates the units of measurement).

To select/deselect the columns that should be displayed in the Region Report:

- 1 Choose **Select Columns**.

A dialog box appears displaying two lists (**Show Columns/Hide Columns**).

- 2 Select the columns you want to show or hide and click the appropriate arrow symbol to transfer the items between the two lists.

3 To deselect a particular item, just click it again.

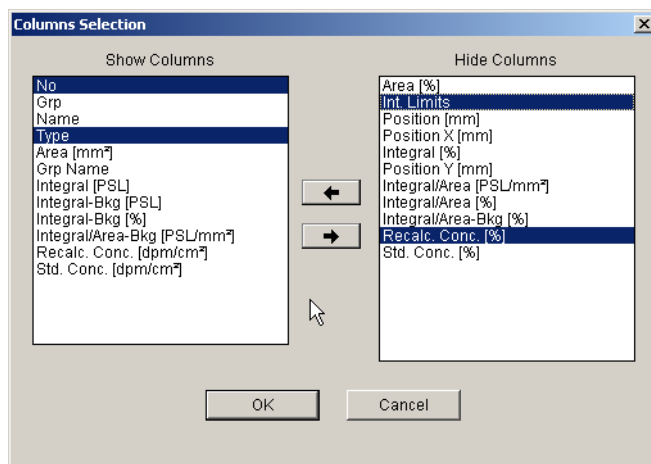


Figure 2-11 Columns Selection Dialog Box

In addition, each cell of the **Region Report** has its own contextual menu attached, providing commands for grouping of regions and standards as well as displaying/hiding of the special row types **Sum**, **Total**, **Reminder**.

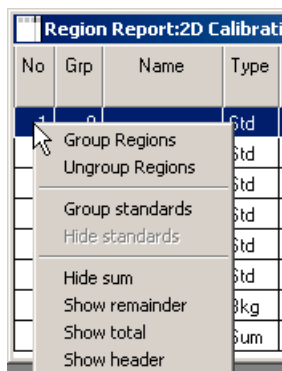


Figure 2-12 Contextual Menu of a Single Region Report Row

If multiple rows are selected, the contextual menu contains only the grouping commands applicable in this context.

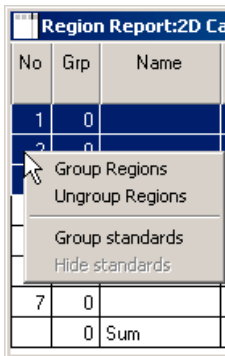


Figure 2-13 Contextual Menu of Region Report Rows (Multiple Selection)

The contextual menus of the **TYPE**, **NAME**, **STD.** ~ and ~ [%] cells provide additional menu items for their respective context:

If a single row is selected, the contextual menu of the **Type** cells contains the additional commands **Set as Normal**, **Set as Background** as well as **Set as Total** for changing the region's type.

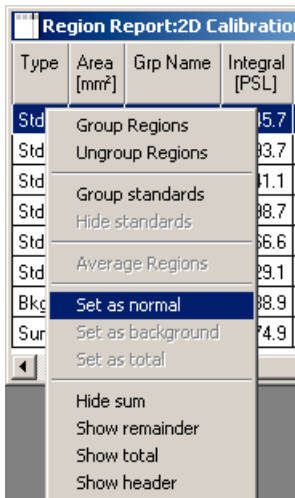


Figure 2-14 Contextual Menu of the Region Report's Type Cell (Single Selection)

If multiple rows are selected, the contextual menu of the **Type** cells contains the additional command **Average Regions**, which inserts an additional row for the average values of regions. The three above-mentioned menu items for changing the region type are unavailable in this context and therefore displayed in gray.

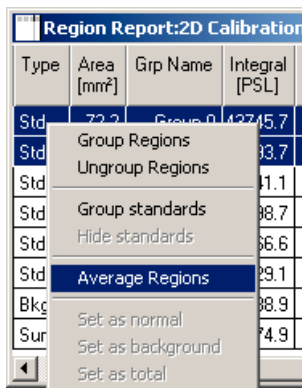


Figure 2-15 Contextual Menu of the Region Report's Type Cell (Multiple Selection)

The contextual menu of the **Name** cells contains the additional command **Change Name** for renaming the selected region.

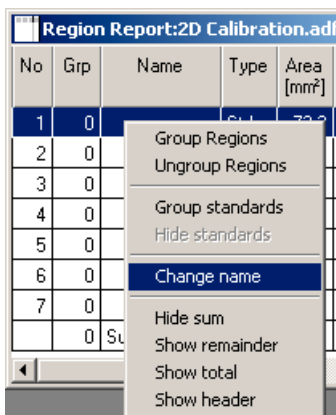


Figure 2-16 Contextual Menu of the Region Report's Name Cell

The additional menu item **Set Standard** of the **Std.~** row cells opens the **Region Calibration** dialog box, allowing you to change the standard value and unit for the selected region.

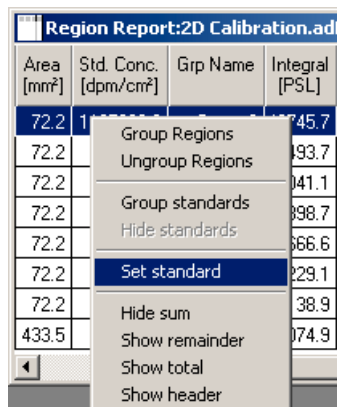


Figure 2-17 Contextual Menu of the Region Report's Std. Row Cell

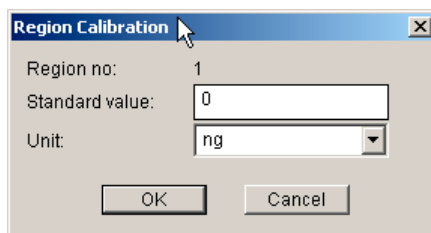
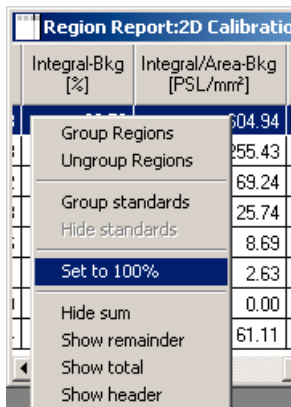


Figure 2-18 Region Calibration Dialog Box

The contextual menu of cells containing percentages provides an additional **Set to 100 %** item, used to set the value of the selected cell to 100 % and recalculating the percentages of all other rows in this column accordingly.



Integral-Bkg [%]	Integral/Area-Bkg [PSL/mm²]
604.94	
255.43	
69.24	
25.74	
8.69	
2.63	
0.00	
61.11	

Figure 2-19 Contextual Menu of the Region Report's % Cells

Region Calibration Window

To open the **Region Calibration** window:

- Choose **Quantity Calibration** from the **View** menu, or
- Double-click the miniaturized calibration curve display on the **Region Calibration** toolbox.

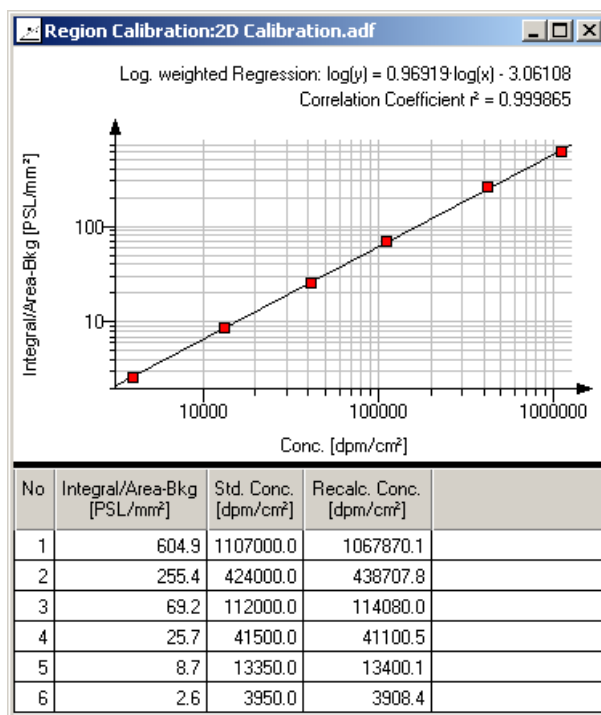


Figure 2-20 Region Calibration Window Example

The **Region Calibration** window is bipartite, the upper pane displays the curve of the calibration function, the underlying function, the correlation coefficient and the x-axis intercept. The lower one contains a table, listing the standards, their given and recalculated standard values etc. Additional columns can be displayed. Just right-click on the table heading and choose the **Select Columns** command from the contextual menu, that appears. The **Columns Selection** dialog box opens, allowing you to select the desired columns from the list.

Graphics Pane of the Regions Calibration Window

The curve shown in the upper part of the **Region Calibration** window in this example displays the background-corrected intensity per area as a function of the quantity (concentration) of the standard.

For a more detailed view of the plot, you can activate the zoom function:

To zoom one of the plot's axis:

- 1 On the toolbar, click the **Select** button.



Figure 2-21 Select Button (Main Toolbar)

- 2 Click and drag one of the axis with the left mouse button pressed.

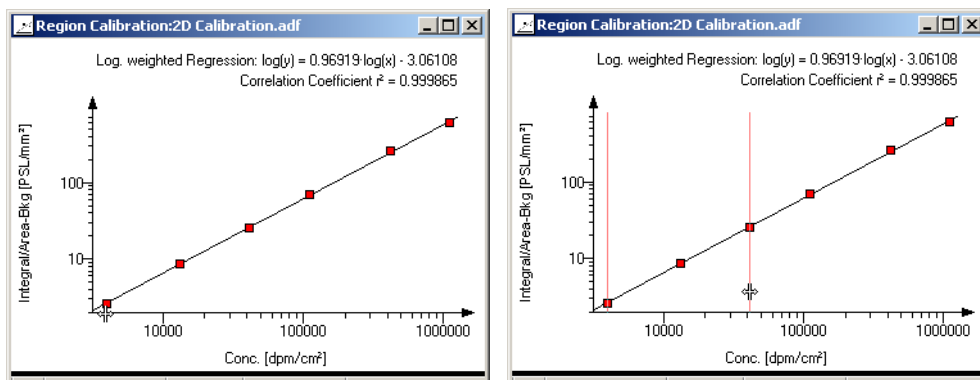


Figure 2-22 Clicking and Dragging the X-Axis



3 Release the button

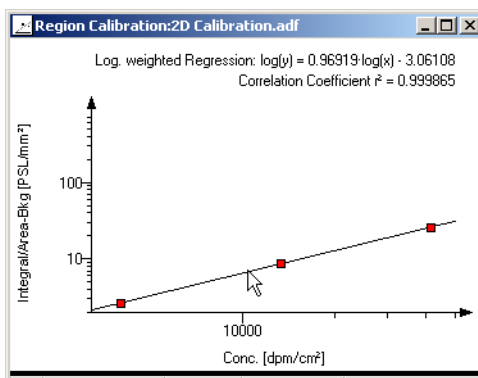


Figure 2-23 Zoomed Graph

To zoom a curve segment:

- 1 Click the **Zoom** button on the toolbar.

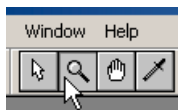


Figure 2-24 Zoom Button (Main Toolbar)

- 2 Draw a rectangle around the area of the plot you want to see in more detail.

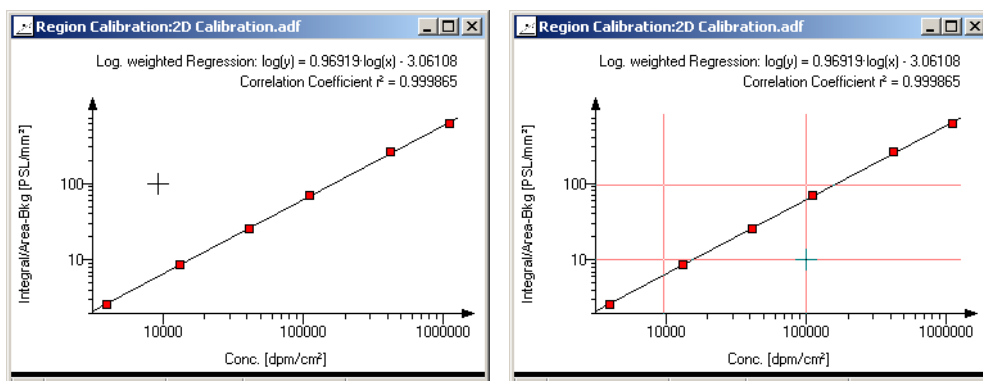


Figure 2-25 Specifying the Area to be Zoomed

3 Release the button.

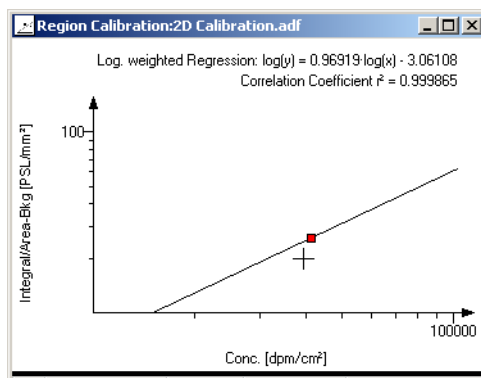


Figure 2-26 Zoomed Curve Segment

Contextual Menu of the Region Calibration Window

The contextual menu of the upper pane of the **Region Calibration** window contains two commands: **Scales** and **Grid**.

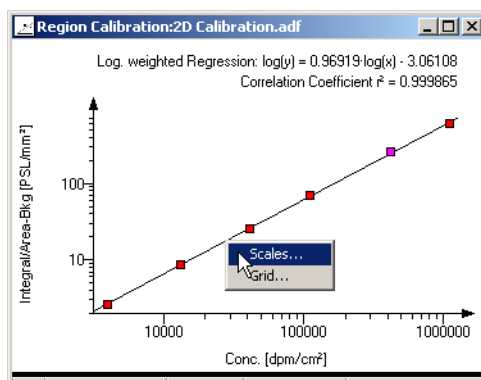


Figure 2-27 Contextual menu of the Region Calibration Window (Graphics Pane)

The **Scales** command allows you to configure the graphical output. After clicking the **Scales** command, the **Data Scaling** dialog box opens. Here the **Minimum** and **Maximum** value of the x- (Concentration) and y-axis (Intensity) can be selected. In addition, you can

turn on logarithmic representation and automatic scaling of either axis separately by clicking the appropriate option (**Logarithmic** and **Automatic**) below the **Minimum** and **Maximum** spin boxes.

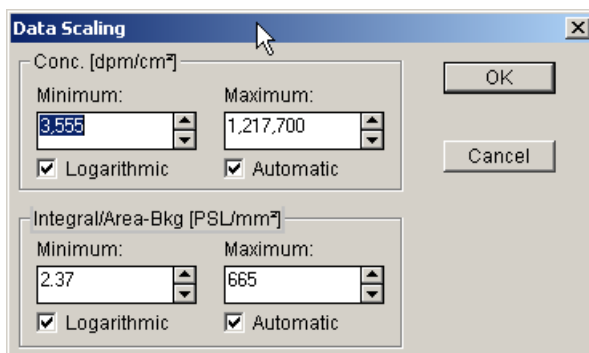


Figure 2-28 Data Scaling Dialog Box (Region Calibration Window)

The **Grid** command opens the **Grid** dialog box, which allows you to lay grids over both axis that are an extension of either the big or the small ticks on the axes.

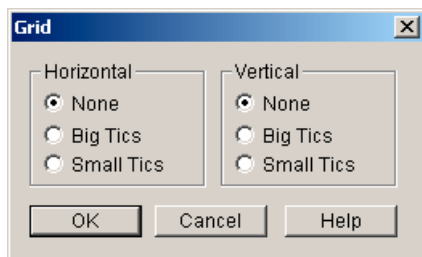
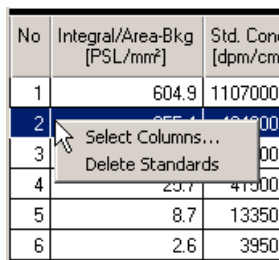


Figure 2-29 Grid Dialog Box

The table in the lower part of the **Region Calibration** window provides its own contextual menu:



No	Integral/Area-Bkg [PSL/mm²]	Std. Conc [dpm/cm²]
1	604.9	1107000.
2	855.1	1616000.
3		00.
4	23.7	41500.
5	8.7	13350.
6	2.6	3950.

Figure 2-30 Contextual Menu of the Region Calibration Window (Table Pane)

The column headings are equipped with the standard contextual menus described in the “Region Report Window” section of this manual.

If you right-click on any of the table rows, the row's contextual menu opens, allowing you to **Select Columns** for displaying or **Delete Standards** from the table.

The following columns are available:

Table 2-3 Columns Available in the Region Calibration Window

Columns	Short Description
No	The number of the region
Name	The name of the region as it was assigned by the user.

Quantity Calibration

Integral-Bkg [*signal unit*]

The background intensity is subtracted from the calculated intensity of the region to result in the signal intensity of the region. For each group of regions the background is determined in the following order: the local background area around the region, or the local background defined for the group, or the general background defined in group 0, whatever is available.

signal unit and *length unit* = units of measurement, depending on the type of the imaging device used to obtain the image and the options set in the **Image Attributes** dialog box.

concentration unit and *quantity unit* = unit of measurement, specified by the user through calibration

unit = quantity or concentration unit of measurement, depending on the argument



Table 2-3 Columns Available in the Region Calibration Window

Columns	Short Description
Std. Quantity [<i>quantity unit</i>]	The quantity of the region, as entered for the calculation of the standard curve. With the Quantity Standards the background corrected Intensity values of the regions are calibrated.
Recalc. Quantity [<i>quantity unit</i>]	A calculation of the quantity in the region, based on the current calibration curve.
Concentration Calibration	
Integral / Area – Bkg [<i>signal unit</i> / <i>length unit</i> ²]	The background intensity is subtracted from the calculated intensity of the region and this signal intensity is divided by the area.
Std. Conc. [<i>concentration unit</i>]	The concentration of the region, as entered for the calculation of the standard curve. With the Concentration Standards the background-corrected Intensity per Area values of the regions are calibrated
Recalc. Conc. [<i>concentration unit</i>]	A calculation of the concentration in the region, based on the current calibration curve.
Difference [<i>unit</i>]	The difference without sign between the entered standard and the recalculated value.
Rel. Deviation [%]	The relative difference with sign of the entered standard and the recalculated value compared to the standard value (= Deviation / Std. Conc. [Std. Quantity]).
Deviation [<i>unit</i>]	The difference with sign between the entered standard and the recalculated value (Recalc. Conc. [Recalc. Quantity] – Std. Conc. [Std. Quantity]).
Rel. Error [%]	The relative difference without sign of the entered standard and the recalculated value compared to the standard value (= Difference / Std. Conc. [Std. Quantity]).
<i>signal unit</i> and <i>length unit</i> = units of measurement, depending on the type of the imaging device used to obtain the image and the options set in the Image Attributes dialog box.	
<i>concentration unit</i> and <i>quantity unit</i> = unit of measurement, specified by the user through calibration	
<i>unit</i> = quantity or concentration unit of measurement, depending on the argument	

Additional Toolboxes in 2D Densitometry

In addition to the standard AIDA toolboxes **Display Control** and **Publishing Layout**, the following toolboxes become available after selecting the **2D Densitometry** mode.

- Region Determination
- Region Calibration

You can access these additional toolboxes from the **Evaluation** menu or by clicking the appropriate button on the toolbar.

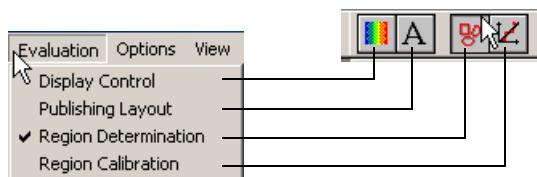


Figure 2-31 Mapping between Evaluation Menu Entries and Toolbar Buttons

Region Determination Toolbox

The controls of the **Region Determination** toolbox allow you to define regions in the image for signal intensity evaluation.

To display the Lane & Peak Determination toolbox:

- On the main menu, click **Evaluation** and choose the **Region Determination** entry.
or
- Click the **Region Determination** button on the toolbar.

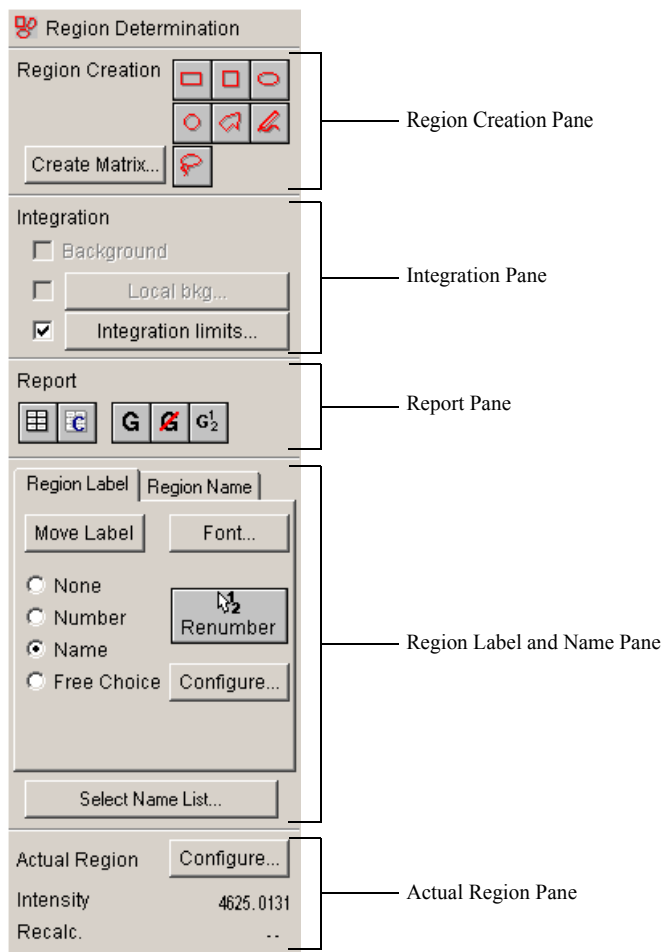


Figure 2-32 Region Determination Toolbox (Overview)

Region Creation Pane

The **Region Creation** pane of the toolbox is used to mark various areas in the image.

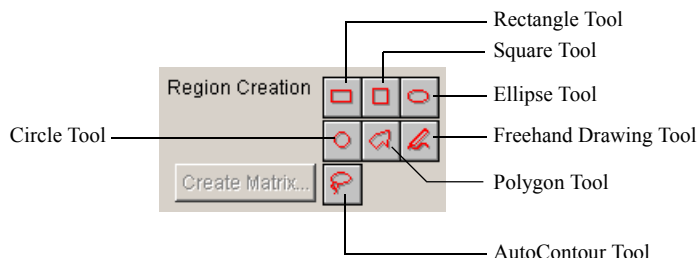


Figure 2-33 Region Creation Pane (Overview)

Areas of different shapes (rectangles, squares, ellipses and circles) can be marked.

To create a region:

- 1 Click on the symbol of the desired shape.
- 2 Click at the upper left corner of the area, hold down the mouse button and drag it to the desired lower right end of the shape.
- 3 Release the mouse button.

The area can be reshaped by moving the handles .

Clicking within the area changes the handles to this symbol , now the object can be rotated around its center. Clicking within the area again changes the handles back. When the overlay has received its final shape and tilt, right-click or press the ENTER key to save the object and go on with the next action.

Polygon Tool: This Tool allows you to draw an enclosure by clicking at multiple subsequent points surrounding the object and finalizing the encircling with a double-click.

Freehand Drawing Tool: Click at a start point and drawing the outside shape of the object while holding down the mouse button. On release, the object is closed automatically.

The areas can be changed in size by moving the handles around.

The corner handles are used for total size change, the side handles are used for size changes in the direction of the handle. Clicking in the middle of the object switches to the turning mode as outlined above. A third click will switch to the re-shape modus where all edges of the object are shown with an individual handle.

The **AutoContour Tool** connects pixels of similar intensity to encircle an object automatically:

- 1 After activating the function, click on the border area of a region within the image that you would like to encircle and drag the mouse pointer slowly to the side of the object.
- 2 Try to drag the cursor around (with the mouse button pressed) until the area is completely encircled.
- 3 Release the mouse button.
- 4 Remember to press ENTER or the right mouse button after the area has been correctly assigned.

To place multiple identical objects on top of the image, use the **Create Matrix** button to open the **Matrix Options** dialog box.

This function is useful for the analysis of multi-well plates or dot blots where signals of identical shape and distance have to be analyzed.

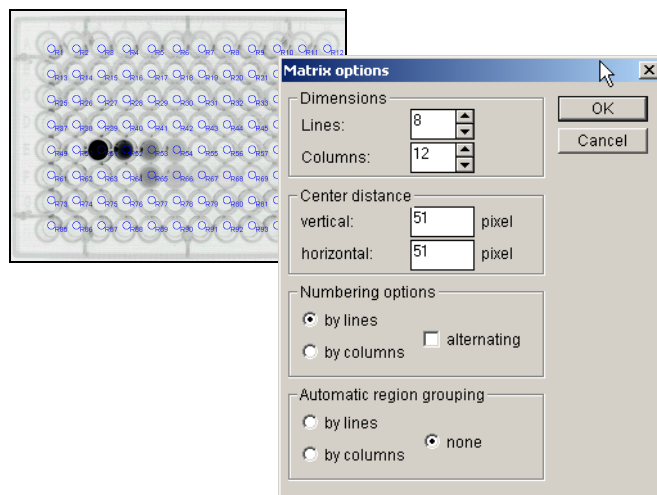


Figure 2-34 Matrix Options Dialog Box With Application Example

Dimension: In the **Dimension** pane of the **Matrix Options** dialog box you can enter the number of objects to be created in x- (**Lines**) and y- (**Column**) direction of the matrix.

Center Distance: In the **Center Distance** part of the dialog box enter the **Vertical** and **Horizontal** distance (mid to mid) between objects in the matrix.

Numbering Options: The regions in the matrix can be numbered **By Lines** (from left to right) or **By Column** (from top to bottom). Clicking the **Alternating** option results in numbering the first line from left to right, the second line from right to left and so on. The **Alternating** numbering is also available for columns.

Automatic Region Grouping: The resulting regions can also be grouped together **By Lines** or **By Columns** if they represent multiple independent analysis points for example.

Integration Pane

The **Integration** pane of the **Region Determination** toolbox is used to define the background calculation for the integration of regions. Any region that is marked with the **Background** option turned on is taken as an example for the background signal. Multiple background areas can be assigned and will be averaged.

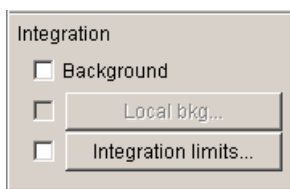


Figure 2-35 Integration Pane

To assign a frame around the active region as a local background, select the checkbox next to the **Local Bkg** button. The general background calculated by the **Background** function is ignored for regions that have a local background assigned to them. This function is not advised for blurred spots of usual 2D gel applications because part of the

actual sample might be mistaken for local background. Clicking the **Local Bkg.** button opens the **Local Bkg Settings** dialog box, where a shape and size (in pixels) can be selected for the local background frame.

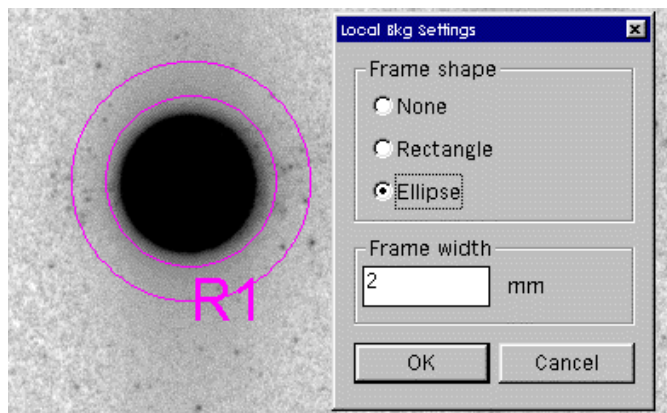


Figure 2-36 Local Bkg Settings Dialog Box

The **Integration Limits** option of the **Integration** pane allows you to integrate only those pixels that lie within a defined intensity range. Thus a region that has some noise (dirt) on it can be integrated without the unwanted signal. The intensity borders can be assigned via the **Change Integration Limits** dialog box.

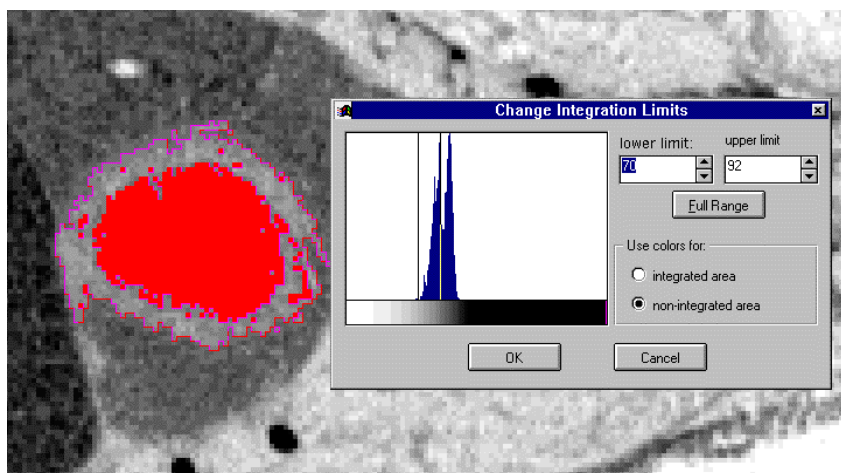


Figure 2-37 Change Integration Limits Dialog Box

The intensity histogram is used to set the lower (left) and upper (right) intensity levels that will be used for the integration. This is done by dragging the vertical black lines from the right and left end of the histogram to the desired position, or by entering the appropriate values in the designated fields (**Lower Limit**, **Upper Limit**). As a consequence the pixels that have a lower intensity or a higher intensity than the designated integration area (peaks on the left or right of the margins shown in the example) will not be used for integration. The choice of coloring the **Integrated Area** or the **Non-integrated** area to visualize the effect can be selected in the **Use Colors for** field.

Report Pane

The **Report** pane of the **Region Determination** toolbox is used to activate and configure the report that summarizes the integration results.

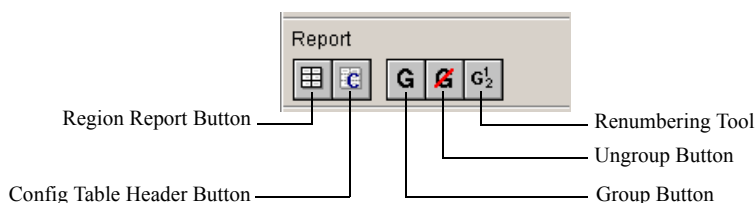


Figure 2-38 Report Pane

Clicking the **Region Report** button activates the display of the report (same as the **Region Report** function in the **View** menu).

With the **Config Table Header** button the table header name for the columns **Integral**, **Integral/Area**, **Quantity** can be configured.

The following three buttons are used to group, ungroup and renumber (in this order) groups and regions. Every area can only belong to one group. Grouped areas will get a common summary line at the end of all integrated regions in the region report:

Groups can be formed by selecting multiple regions and clicking the **Group** button.

Selected regions can be removed from their group by selecting the **Ungroup** button.

Using the **Renumbering Tool**, all groups are renumbered in sequential order. This avoids gaps in numbers which are caused by ungroup processes.

Region Label and Name Pane

The **Region Label and Name** pane is used to control the labeling and name of regions.

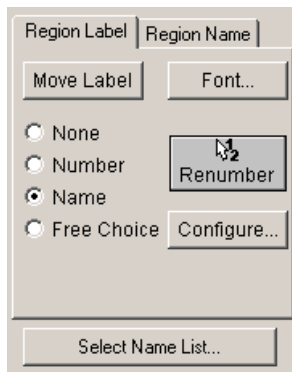


Figure 2-39 Region Label and Name Pane

Region Label tab: You can turn off labeling (**None** radio button), select a number as current label (**Number** radio button) or display the name of the region instead (**Name** radio button). Alternatively you can select a label freely from the column list displayed after clicking the **Free Choice** option and then clicking the **Configure** button. The contents of this list is identical to the one from the **Columns Selection** dialog box (cf. the Region Report pane of this manual). You can change the position of a region label using the **Move Label** button to increase visibility:

To move a label to a desired destination:

- 1 Select the region with a mouse click.

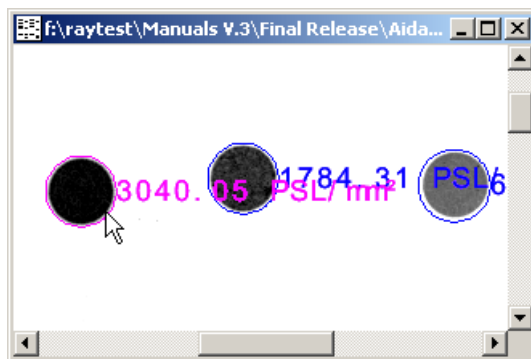


Figure 2-40 Selecting Region

- 2 Click the **Move Label** button on the **Region Label and Name** pane of the **Region Determination** toolbox.

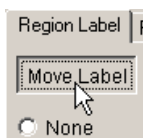


Figure 2-41 Move Label Button

The label of the previously selected region turns its color from magenta to red.

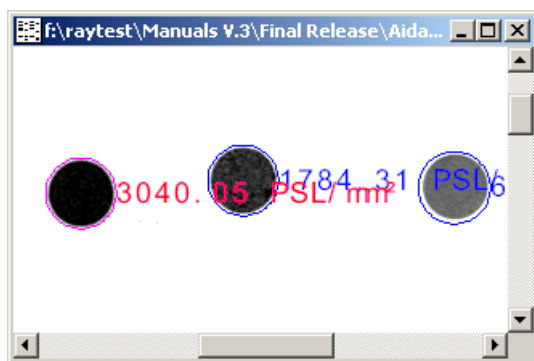


Figure 2-42 Activated Label

- 3 Drag the label to its designated location.

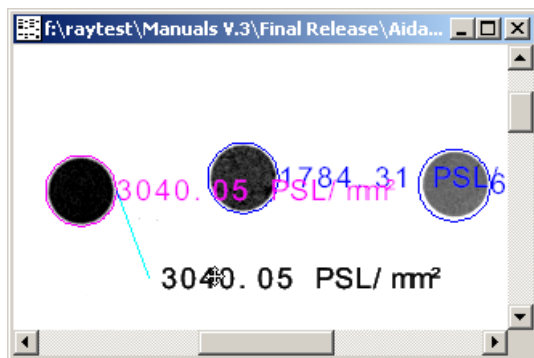


Figure 2-43 Moving Label

- 4 Confirm the action by clicking the right mouse button, or alternatively by pressing the ENTER key on your keyboard.

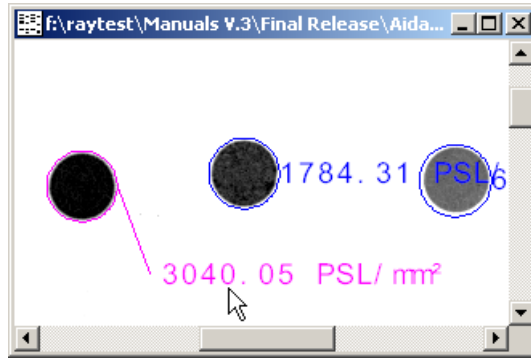


Figure 2-44 Label Repositioning Confirmed

You can select the **Font** type and **Size** of the characters as well as their font style (bold (**B**), italic (**I**) and roman typeface on white background) using the **Font** button. The **Choose Font** dialog box is displayed, allowing you to specify the font options for the label.

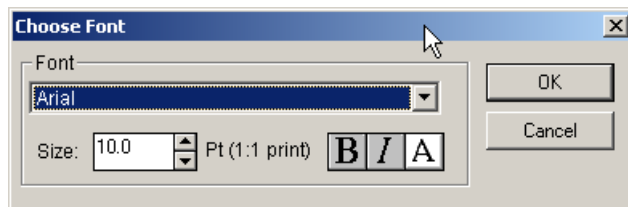


Figure 2-45 Choosing Font Properties

The regions can be renumbered if necessary by activating the **Renumber** function. This function opens the **Renumber Regions** dialog box. The numbers are displayed subsequently in a combo box and can be assigned to the desired regions by simply clicking on them.

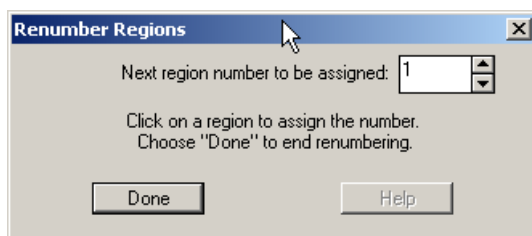


Figure 2-46 Renumber Regions Dialog Box

The **Region Name** tab controls are used to assign names to the various regions.

To assign names to regions:

- 1 Select a region on the image.
- 2 Click the **Region Name** tab.

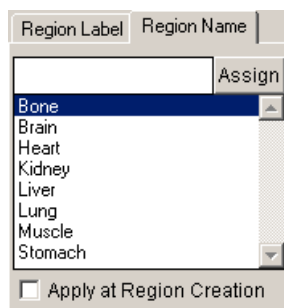


Figure 2-47 Region Name Tab

Common names can be entered via a text box into a list that can facilitate the designation of redundant names ('Negative Control', 'Positive Control', 'Standard', 'Background', etc.).

- 3 Type a name in the text box or select one from the list below the text box.
- 4 Click **Assign**.

- 5 (Optional) Click the **Apply at Region Creation** option to assign the names in the list to any newly created region one after another in the order of their appearance in the list.

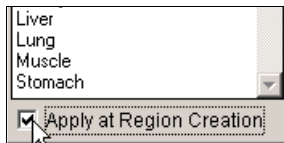


Figure 2-48 Selecting Apply at Region Creation Checkbox

To edit the list of names:

- Click the **Select Name List** button below the **Region Name** tab,

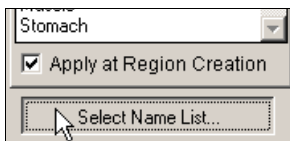


Figure 2-49 Select Name List Button

This opens the **Region Nametables** dialog box.

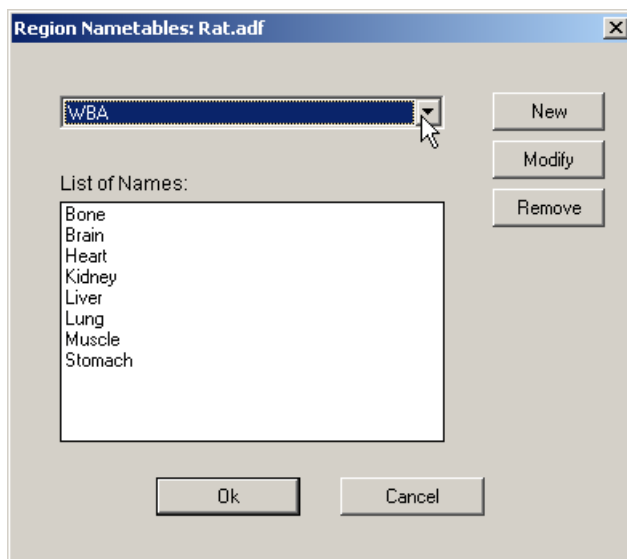


Figure 2-50 Region Nametables Dialog Box

Using this dialog box, you can:

- create a new list of names by clicking the **New** button, or,

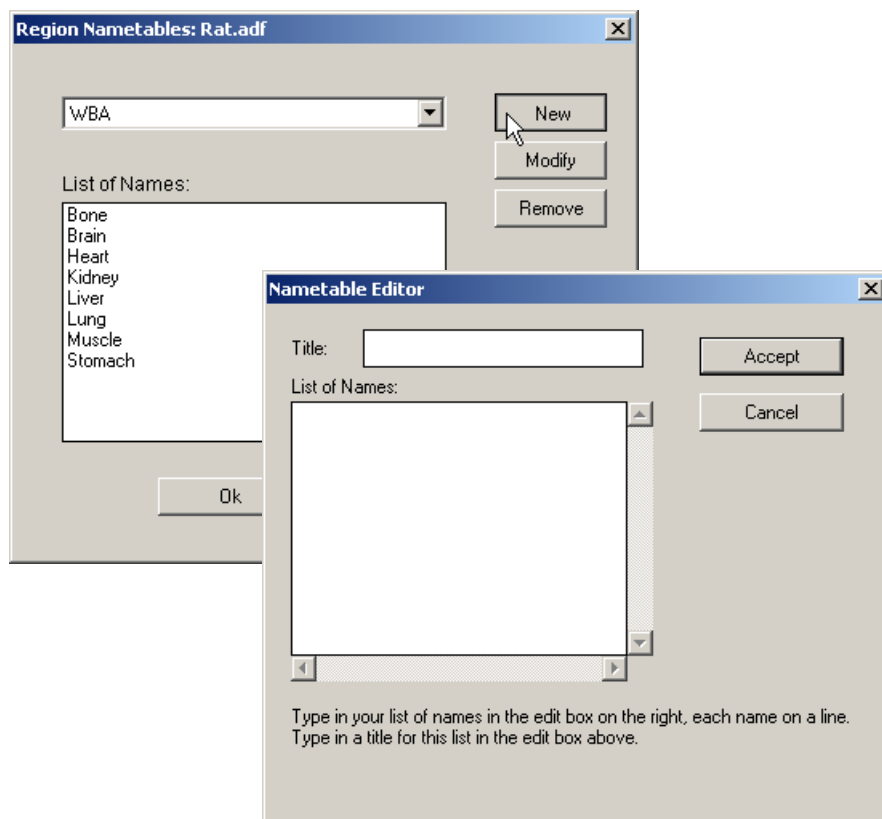


Figure 2-51 Nametable Editor (Creating New List of Names)



- edit an existing name table by choosing **Modify**.

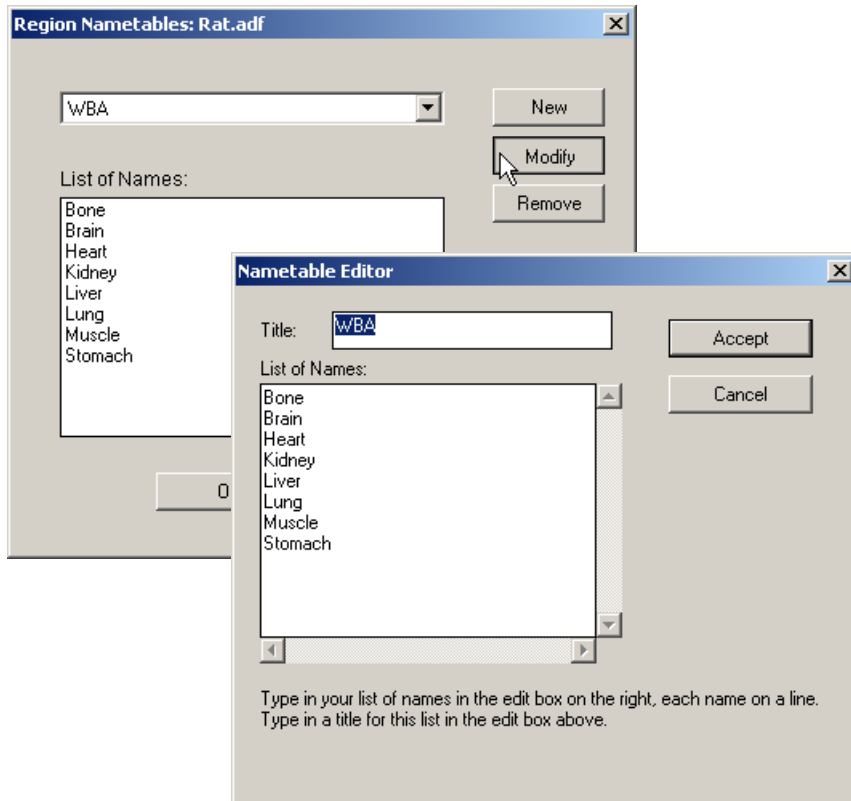


Figure 2-52 Nametable Editor (Editing Existing Name Table)

Actual Region Pane

The **Actual Region** pane provides information about the selected region.

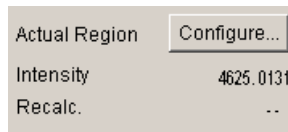


Figure 2-53 Actual Region Pane

Use the **Configure** button to add or delete categories from the list shown below the **Configure** button. This opens the **Column Selection** dialog box described in the Region Report section of this manual.

Region Calibration Toolbox

The controls of the **Region Calibration** toolbox allow you to perform the calibration of the 2D densitometer. For the calibration, reference regions for the creation of standard curves are necessary on the same image..

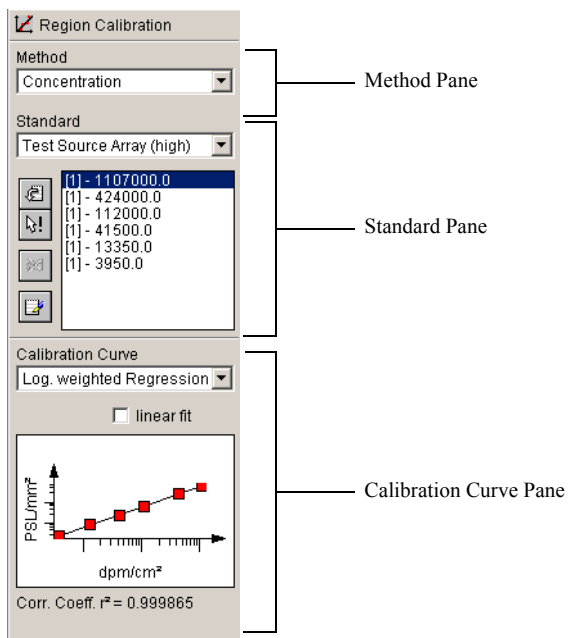


Figure 2-54 Region Calibration Toolbox (Overview)

To display the Region Calibration toolbox:

- On the main menu, click **Evaluation**, and then click **Region Calibration**,
or
- Select the **Region Calibration** button on the toolbar.

Method Pane

The drop-down menu of the **Method** control pane is used to specify the calibration method (**Quantity** or **Concentration**).

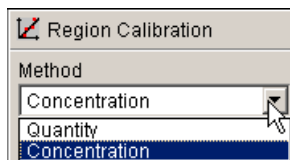


Figure 2-55 Method Drop-Down Menu

Selecting **Quantity** will result in the analysis of integrated intensities of all pixels in the defined region whereas **Concentration** will compare the average intensity in the regions.

Standard Pane

Using the controls of the **Standard** pane you can assign standards to selected regions.

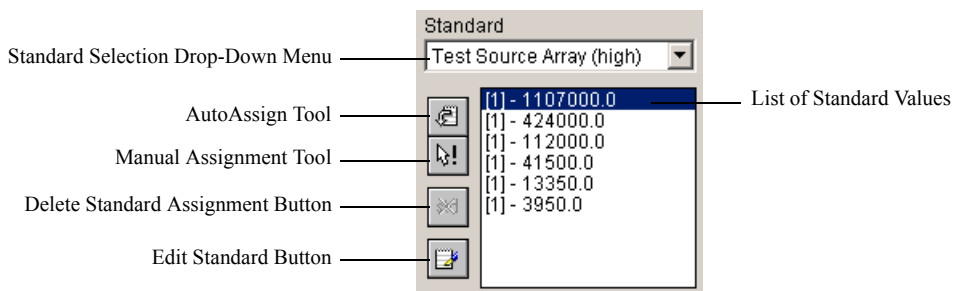


Figure 2-56 Standard Pane

From the **Standard** drop-down menu a predefined list of standard values can be selected. The specified standard values are displayed in the pane below the **Standard** drop-down menu.

Beside this pane there are four buttons:

AutoAssign Tool: This button allows you to subsequently assign all values in the list by clicking with the mouse on the appropriate region. Thus the first value (1107000.0) is assigned to the first clicked region, the second value (424000.0) to the secondly clicked region and so on.

Manual Assignment Tool: The second button allows you to assign any value to the clicked region by entering the appropriate value and unit for the selected region in the **Region Calibration** dialog box that appears.

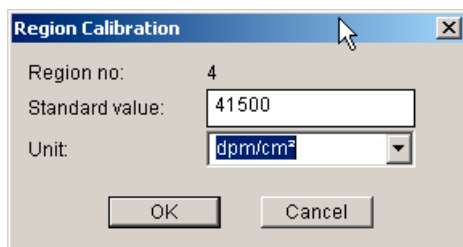


Figure 2-57 Region Calibration Dialog Box

Delete Standard Assignment Button: By clicking this button, all calibration values of selected regions can be canceled.

Edit Standard Button: The last of the four buttons is used to enter new lists of standard values. Clicking this button opens the **Quantity Standards** (or **Concentration Standards**) dialog box, which is used to enter a new list or to modify an existing one. To create a new list, name the list in the **Standard** text box and specify its **Values**, **Unit** and **Precision** (displayed fraction digits).

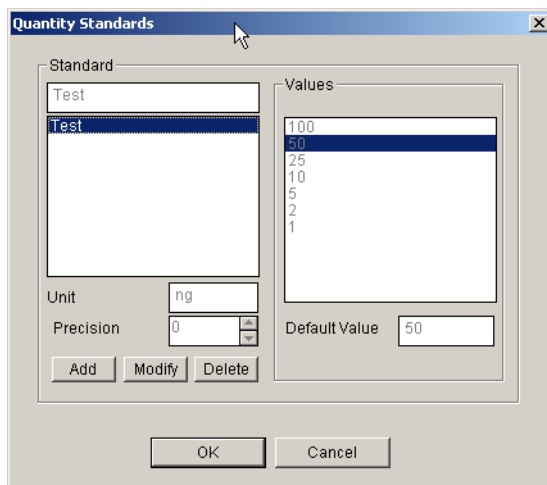


Figure 2-58 Quantity Standards Dialog Box

The **Add**, **Modify** and **Delete** buttons are used to alter lists in the same box.

All used standards should be saved for future use. This is also true for one-time standards because evaluation get frequently re-done, which means that the standard values are lost unless entered in the list.

Calibration Curve Pane

In the **Calibration Curve** pane of the **Region Calibration** toolbox the mathematical calculation of the standard curve can be selected and the result is displayed in the graph at the lower end of the toolbox.

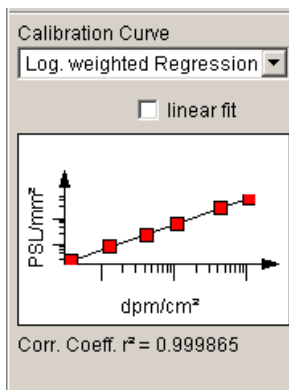


Figure 2-59 Calibration Curve Pane

The **Calibration Curve** drop-down menu provides the following functions:

- Linear Interpolation
- Linear Regression
- Logarithmic Regression
- Log. Weighted Regression

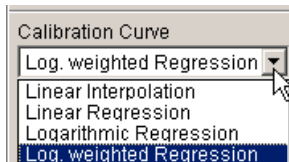


Figure 2-60 Selecting Calibration Curve Type

Linear Interpolation calculates lines to join each data point.

Linear Regression optimizes a linear graph so that the deviation of the actual points from the curve is minimized.

Logarithmic Regression is used to determine the standard curve with logarithmic characteristics, which is found in many saturating effects (e.g. absorption).

Log. Weighted Regression can be described as linear regression using a double logarithmic scaling. The result is a better optimization of the curve fit for the smaller values, instead of a better fit for the higher values (**Linear Regression**).

If you choose **Linear Interpolation**, **Linear Regression** or **Logarithmic Regression**, you can select the **(0,0) Origin** option to force the curve through the origin. For **Log.**

Weighted Regression select the **Linear fit** option to force the curve to a linear function.

The correlation coefficient (**Corr. Coeff.**) of the estimation is shown below the curve. A value of 1 identifies a perfect correlation, a value of 0 indicates full independence of values. Good standard curves should have value very near to 1.

Double-clicking on the graphics pane opens the **Region Calibration** window (see the “Region Calibration Window” section of this manual for details).

Sometimes a warning will be displayed below the graphics pane, stating 'bkg not determined', to indicate that no background region has been assigned yet. It can also indicate the lack of a local background for some regions.

Publishing Layout Toolbox in 2D Densitometry

In AIDA 2D Densitometry the standard AIDA **Publishing Layout** toolbox is expanded by two additional buttons for specifying the print options for the **Region Report** and **Region Calibration**.

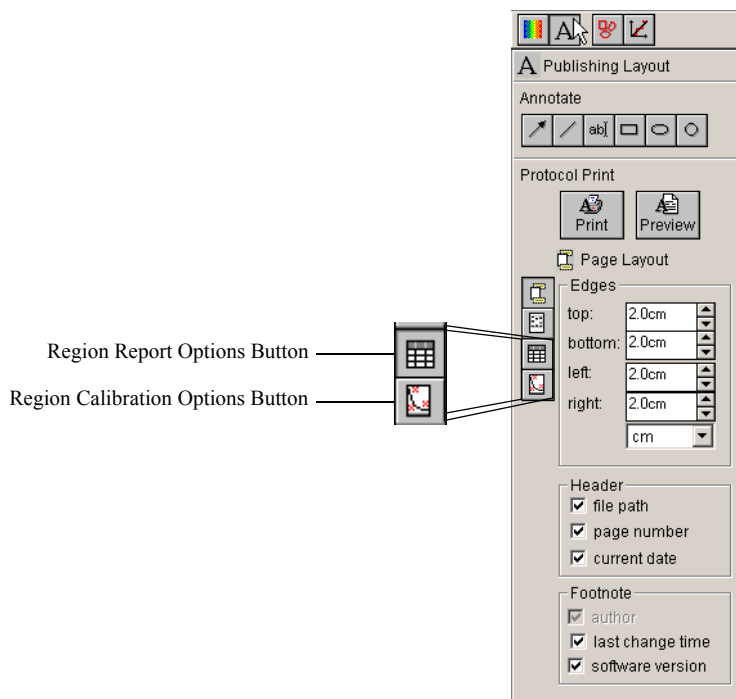


Figure 2-61 Publishing Layout Toolbox (Overview and Toolbar)

Print Options for Region Report

With the first one you can configure layout setting for the **Region Report** window in the **Protocol Print** pane.

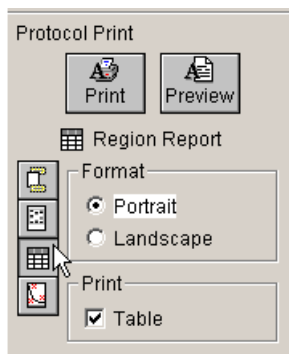


Figure 2-62 Print Options for the Region Report Window

Here you can select the paper orientation (**Portrait** or **Landscape**) by clicking the appropriate radio button in the **Format** pane. In the **Print** pane the scope for the print output can be specified. To turn on printing of the **Region Report** table, click the **Table** box.

Print Options for Region Calibration

The second additional button of the **Publishing Layout** toolbox is used to configure the layout setting for the **Region Calibration** window.

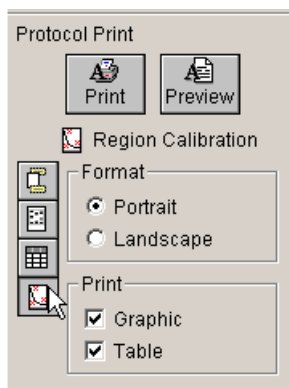


Figure 2-63 Print Option for the Region Calibration Window

You can select the paper orientation (**Portrait** or **Landscape**) by clicking the appropriate radio button in the **Format** pane. The options in the **Print** pane enable or disable the printing of the calibration curve (**Graphic**) and/or the calibration table (**Table**).

Print

In AIDA there are two ways for printing your image and evaluation data:

- Choose **Print** from the **File** menu,
- or*
- Use the **Protocol Print** pane on the **Publishing Layout** toolbox.

The **Print** command of the **File** menu allows you to print your data 'on the fly' without permanently storing the layout/printing settings, whereas the **Protocol Print** pane provides you with a complete set of printing features which includes saving the setting with the data file for reasons of reproducibility.

Choosing the **Print** command from the **File** menu opens the **Print** dialog box, allowing you to configure the printout to contain only the overlays, results and graphs that are currently needed. Click the checkboxes for the various items to turn them on or off. Select the number of copies, you want to print.

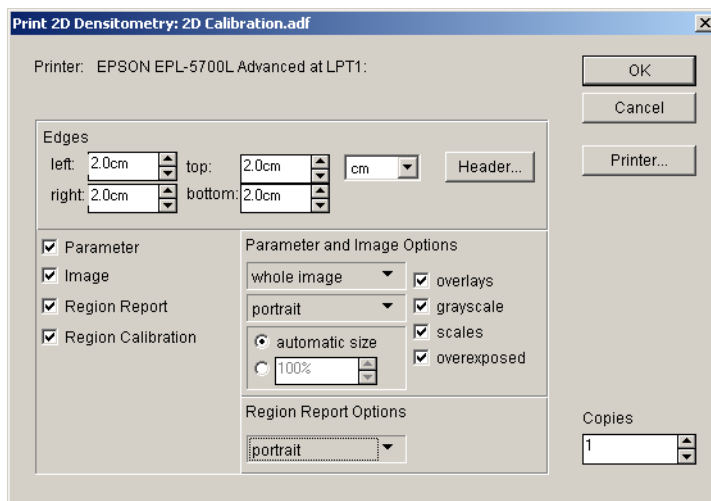


Figure 2-64 Print Dialog Box

The margins of the printout can be selected in the **Edges** part of the dialog box. The **Header** button displays the following dialog box:

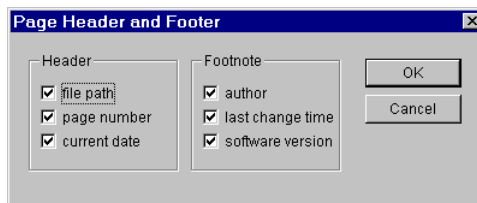


Figure 2-65 Page Header and Footer Dialog Box

Here the **Header** and **Footer** of the report can be configured to contain various image and evaluation specific data.

The **Parameter** (data overlays), the **Image**, **Region Report** and **Region Calibration** information can be selected in the left part of the **Print** dialog box.

In the right part of the dialog box (**Parameter and Image Options**) you can select **Whole Image**, or **Window Content** from the first drop-down menu. **Landscape** or **Portrait** format can be selected from the second drop-down menu. The printing of **Overlays**, **Grayscales**, **Scales**, and **Overexposed** can be selected. The printout can be automatically fitted to the page, or the relative size of the image representation can be selected (**in %**).

In the **Profile Options** pane you can specify to print the **Current Selection** or **all profiles** as well as the paper orientation (**Landscape** or **Portrait**) for the printout.

Preview

As for printing, in AIDA there are two ways of previewing pages before they are actually printed:

- Using the **Print Preview** command from the **File** menu, or
- Using the **Preview** button on the **Publishing Layout** toolbox.

The **Print Preview** command of the **File** menu opens the **Preview** dialog box, which allows you to specify the layout settings for the preview. The options presented here are the same as in the **Print** dialog box and those selectable from the various panes of the **Publishing Layout** toolbox. However, they are not stored with the data file.



Aida/Whole Body Autoradiography (GLP) - Preview :Ra.dad

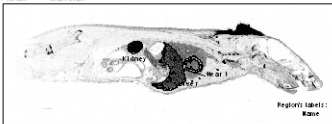
File Edit Evaluation Options View Window Help

Page 1 of 2

Whole Body Autoradiography (GLP) Monday, February 12, 2001, 10:50:10 PM
e:\Program Files\scpi\Whole Image Analyzer\dev3.10.020\Wholeimg\Ra.dad (modified) Page 1 of 2

Parameters

Parameter file: e:\Program Files\scpi\Whole Image Analyzer\dev3.10.020\Example\ra.par
Image data file: e:\Program Files\scpi\Whole Image Analyzer\dev3.10.020\Example\ra.img
Original file: ra
Scan date: Tuesday, April 20, 1993, 10:42:40 AM
Imaging plate type: 2470
Source: Pu-240 1000
Scatterity: 4
Latitude: 4
Digital resolution: 256, 8 bit per pixel
Image size: 4096x, 80 (lower)100 (upper)
Pixel size: 200umX200um



Regions labels:
None

2D Region Report

No	Name	Type	Geo Name	Area (mm ²)	Integral (DPMs)	Integral/Box (DPMs/cm ²)	Integral/Box (DPMs/cm ²)	Relative Count/cm ²	Org. No.
1	bone	Contour	Contour 1	219.24	4602.0171	4602.0171	97.39	21.0001	int. box
2	heart	Contour	Contour 1	38.24	353.2263	353.2263	9.24	9.2401	int. box
3	kidney	Contour	Contour 1	42.92	380.5254	380.5254	27.55	49.1174	int. box
4	liver	Contour	Contour 1	263.36	6871.6227	6871.6227	108.36	22.0379	int. box

Author: Ops Inc. Adalberto Baker
Last Changed: Monday, February 12, 2001, 10:43:40 PM Aida Development Version 3.10.020

Page 2 of 2

Whole Body Autoradiography (GLP) Monday, February 12, 2001, 10:50:10 PM
e:\Program Files\scpi\Whole Image Analyzer\dev3.10.020\Wholeimg\Ra.dad (modified) Page 2 of 2

AutoT Trail Configuration

Image attributes	yes/no	Acceleration	no/yes
Image statistics	yes/no	Modality	yes/no
Region counts	no/yes	Delta	yes/no
Modality	yes/no	Calibration method	yes/no
Delta	yes/no	Calibration equation	yes/no
Background settings	no/yes	Standardize	no/yes
Integration limits	no/yes	Modality	yes/no
Region groups	no/yes	Delta	yes/no
Region markers	no/yes		
Region names	no/yes		

Print only for "Standard", second only for "Change Attribute"

Auto Trail

1. Monday, February 12, 2001, 10:50:38 PM
Whole Body Autoradiography (GLP) (modified) 

Author: Ops Inc. Adalberto Baker
Last Changed: Monday, February 12, 2001, 10:43:40 PM Aida Development Version 3.10.020

Figure 2-66 Preview Example

Export and Import

In AIDA 2D Densitometry various tables and graphs can be exported to be imported in other documents or programs. In addition the calibrations and templates can be exported to be applied to other images. The various functions can be found in the **Export** and **Import** submenus of the **File** menu.

Exporting Templates

To export your own evaluation templates:

- 1 On the **File** menu, click the **Export** menu item and then choose **Template** from the submenu.
- 2 In the **Export Template** dialog box that appears type a name for the export file and confirm the action by clicking **Save**,
or
 - 1 Click the **Export Template** button on the toolbar to open the **Export Template** dialog box

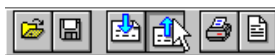


Figure 2-67 Main Toolbar (Export Template Button)

- 2 Type a name for the export file in the **File Name** text box and complete the export action by clicking **Save**.

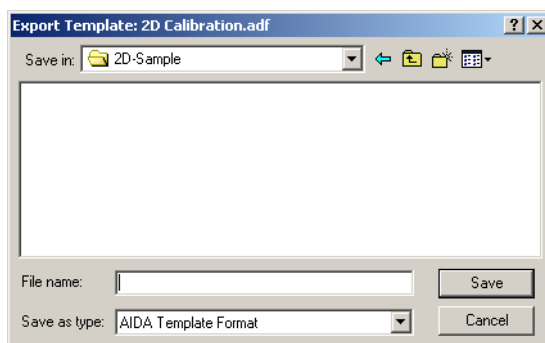


Figure 2-68 Export Template Dialog Box

Exporting Region Report

To export the Region Report:

- 1 On the **File** menu, click the **Export** menu item and then choose **2D Region Report** from the submenu.
- 2 In the **2D Region Report Export** dialog box that appears type a name for the export file.
- 3 Click **Save** to confirm the export.

The **2D Region Report Export Options** dialog box appears, allowing you to set further option for the export of the result table.

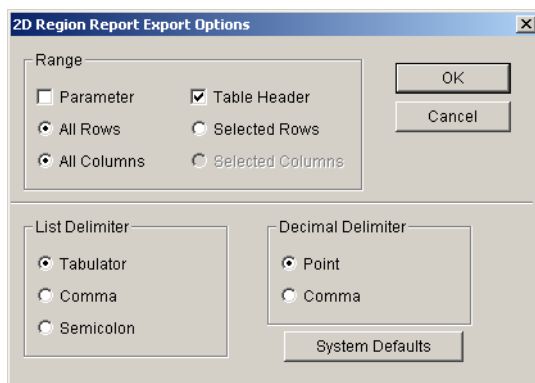


Figure 2-69 2D Region Report Export Dialog Box

You can specify the **Range** of the exported rows and columns (**Table Header**, **All Rows/Columns displayed** in the Region Report window or only the **Selected (highlighted) Rows/Columns** in the Region Report window) as well as the **List Delimiter** and **Decimal Delimiter** in the respective panes of the dialog box. Selecting the **System Defaults** button applies the Windows default settings to **List Delimiter** and **Decimal Delimiter**.

In addition, the parameter settings for the evaluation can be exported by checking the **Parameter** option. If this option is enabled, the following parameter information will be written to the export file:

Table 2-4 Exported Parameter Information

Parameter	Description
<i>Export Parameters</i>	
[1. Row]	Application name (e.g., “”)
[2. Row]	Type of exported data (e.g., “2D Region Report”)
Export settings	Identifier for the export parameter settings
Master nametable	Identifier for the master nametable (comprised of the unique AIDA internal identifier and an import identifier and name)
<i>Image File Parameters (for Fuji BAS Scanner)*</i>	
Parameter file	Image parameter file path (.pcb or .inf)
Image data file	Image raw data file path (.img)
Original filename	Name defined during the scan process
Scan date	Date and time of scan
Imaging plate type	Type of the imaging plate used
Scanner	Scanner type (e.g. “Fuji BAS-5000”)
Sensitivity	Sensitivity parameter of scan
Latitude	Latitude parameter of scan
IP Number	ID of IP plate used; entered during the scan process
Project	Project name entered during the scan process
Sample	Sample name entered during the scan process
Exposure	Exposure time of the plate entered during the scan process
Comment	Image and evaluation comments combined
Digital Resolution	Digital resolution of image
Image size	Width and height of image
Pixel size	Spatial resolution of image
Scan operator	Operator of the scan process
<i>Evaluation File Parameters</i>	
Evaluation file	Evaluation file path (.adf)

Table 2-4 Exported Parameter Information

Parameter	Description
Evaluation date	Date and time of evaluation
Evaluation operator	Operator of evaluation

* The actual image file parameters depend on the data file format and version.

```
# Aida Whole Body Autoradiography (GLP)
# 2D Region Report
#
# Export setting: 20021104 110236 3896 (Till, Default Setting 04-11-2002)
# Master nametable: 20021104 142426 7666 (Master Name Table, 2002-08-16 / 35)
#
# Parameter file: i:\Aida Demo\Rat.inf
# Image data file: i:\Aida Demo\Rat.img
# Original file: rat
# Scan date: Tuesday, April 20, 1993, 4:02:43 AM
#
# Imaging plate type: 20*40
# Scanner: Fuji-BAS 1000
# Sensitivity: 10000
# Latitude: 4
#
# Digital resolution: 256, 8 bit per pixel
# Image size: 348*940, 69.60mm*188.00mm
# Pixel size: 200µm*200µm
#
# Evaluation file: i:\Aida Demo\Rat.adf
# Evaluation date: Monday, November 04, 2002, 3:22:42 PM
# Evaluation operator: System Administrator (Administrator)
```

Figure 2-70 Example of Exported Parameter Information

4 Complete the action by clicking **Ok**.

The data are saved to a simple ASCII (text) file.

Exporting Region Calibration

To export Region Calibration Data:

- 1 On the **File** menu, click the **Export** menu item and then choose **Region Calibration** from the submenu.

- 2 In the **Region Calibration Export** dialog box that appears type a name for the export file and specify the data format.

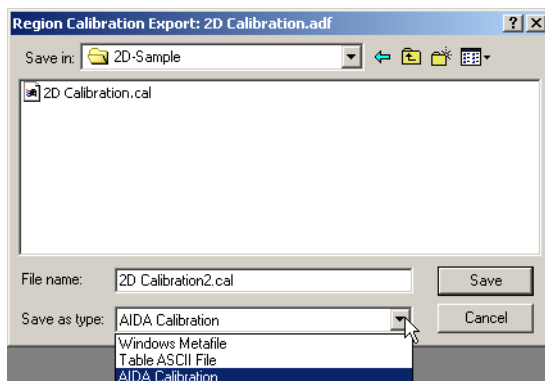


Figure 2-71 Region Calibration Export Dialog Box

To export the graph in the Region Calibration window:

- Choose **Windows Metafile**.

The graph is saved to a separate file in the Windows Metafile format.

To export the result table of the Region Calibration window:

- 1 Select the **Table Ascii File** data format.
- 2 Click **Save** to confirm the export.

The **Quantity Calibration Export Options** dialog box appears, allowing you to set further option for the export of the result table.

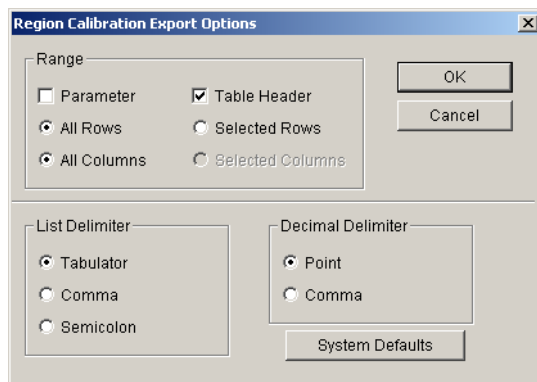


Figure 2-72 Region Calibration Export Options Dialog Box

You can specify the **Range** of the exported rows and columns (**Table Header**, **All Rows/Columns displayed** in the Region Report window or only the **Selected (highlighted) Rows/Columns** in the Region Report window) as well as the **List Delimiter** and **Decimal Delimiter** in the respective panes of the dialog box. Selecting the **System Defaults** button applies the Windows default settings to **List Delimiter** and **Decimal Delimiter**.

In addition, the parameter settings for the evaluation can be exported by checking the **Parameter** option.

- 3 Complete the action by clicking the **OK** button.

The data are saved to a simple ASCII (text) file.

In addition the calibration data can be exported to an AIDA Calibration file that can be imported into another image.

Automatic Export of the Region Reports/Region Calibrations

AIDA's **Automatic Export** option allows you to export 2D Region Reports and/or Region Calibrations automatically when you save an evaluation.

You can specify preferences for the range of the table columns to be automatically exported as well as export settings, which are independent from the current work configurations of the table(s).

The naming of the export files follows the scheme “*Evaluation Name*.Region Report.txt” or “*Evaluation Name*.Region Calibration.txt”, i.e., if the name of the evaluation file is `example.adf`, the Region Report export file has the name `example.Region Report.txt` and the Region Calibration export file is named `example.Region Calibration.txt`. Existing export files are overwritten without prompting the user. However, a backup file of the previous export file version is created.

Automatic Export always exports the entire result table. Restricting the export to selected rows is not possible.

The enabling of the automatic export option as well as its settings are evaluation-specific attributes, which are valid until they are explicitly changed using the **Preferences** or **Automatic Export** options of the **Options** menu or by importing an AIDA template. If you create a new evaluation, the current default configuration for **Automatic Export** is applied and will be saved with the evaluation data in the `adf` file. The configuration remains valid unless it is explicitly changed by the user.

The automatic export settings are timestamped internally. In addition you can add a name and an ID, which are included in the Parameter pane of the export file together with the internal time stamp.

To create automatic export settings:

- 1 From the **Options** menu, choose **Automatic Export**.

The **Automatic Export Setting** dialog box appears.

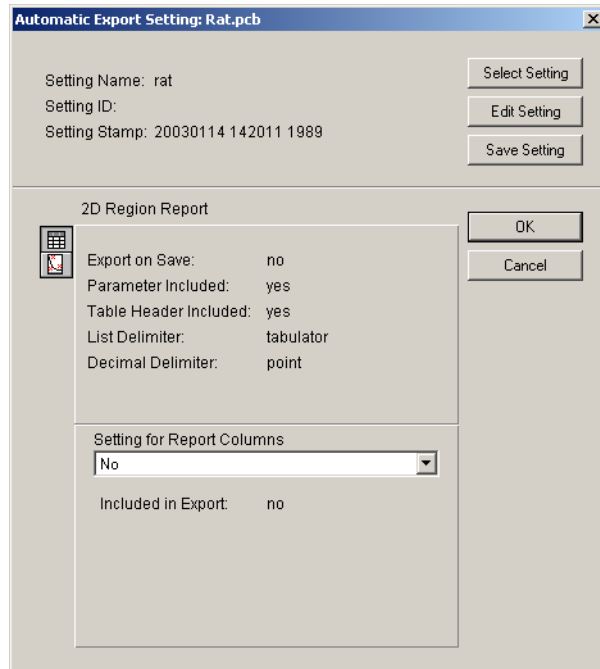


Figure 2-73 Automatic Export Setting Dialog Box

The top pane of the dialog box shows the current settings parameters, whereas the second displays the current settings for the automatic export of the 2D Region Report/Region Calibration. Click on the toolbar to switch between the settings pages.

2 Click **Edit Settings**.

The **Edit Automatic Export Setting** dialog box opens.

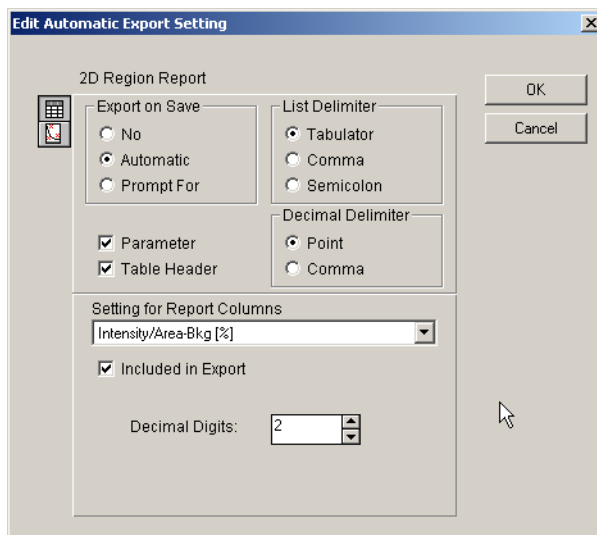


Figure 2-74 Edit Automatic Export Setting Dialog Box

The dialog box provides you with the following option:

- **Export on Save: Automatic** enables the automatic export and generates the export filename automatically from the evaluation filename. **No** disables the automatic export. If you select **Prompt for**, you will be prompted for an export data filename, when you save an evaluation.
- **Parameter:** enables/disables the export of the result table's parameter pane.
- **Table Header:** enables/disables the export of the table header.
- **List Delimiter:** allows you to select a delimiter for the columns
- **Decimal Delimiter:** allows you to select a decimal delimiter format.
- **Settings for Report Columns:** select columns subsequently and click to select or deselect the **Included in Export** option to include/exclude the respective column. Depending on the column you selected, you can specify additional settings (in this example: the **Decimal Digits**) for the exported values.

3 Select the appropriate options and click **OK** to complete the action.

Saving Automatic Export Settings:

- 1 On the **Automatic Export Setting** dialog box, click **Save Setting**.

The **Save Automatic Export Setting** dialog box opens.

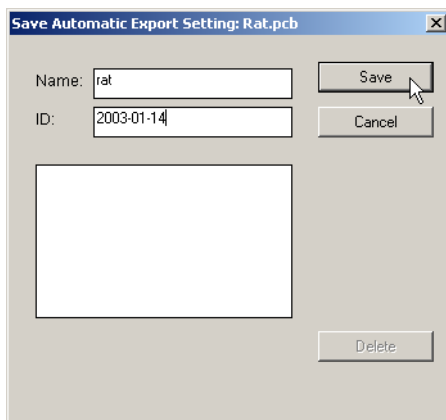


Figure 2-75 Save Automatic Export Setting Dialog Box

- 2 Type a unique name and an ID for the settings and click **Save** to confirm the action.

Selecting Automatic Export Settings

- 1 On the **Automatic Export Setting** dialog box, click **Select Setting**.

The **Select Automatic Export Setting** dialog box appears.

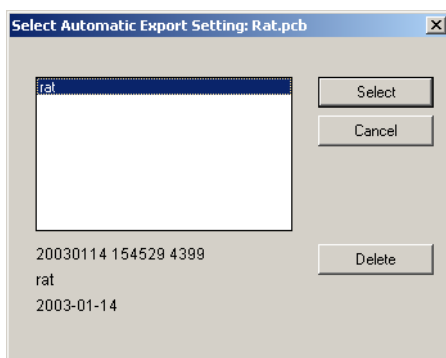


Figure 2-76 Select Automatic Export Setting Dialog Box

- 2 Select a settings set and click **Select** to complete the action.

To delete a settings set:

- 1 Select the set you want to delete in the list of the **Select Automatic Export Setting** dialog box.
- 2 Click **Delete**.

Importing Region Calibration

- 1 To import the calibration curve from another image (e.g. taken at the same time with the same BAS screen, or from a second gel handled at the same time and captured under identical conditions), use the **Import** command of the **File** menu.
The **Region Calibration Import** dialog box appears allowing you to specify the calibration file (.cal).

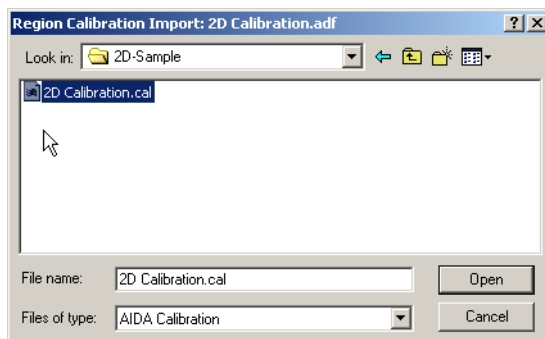


Figure 2-77 Region Calibration Import Dialog Box





Assigning Regions

To define regions for signal intensity evaluation, it is important to set the **Image Attributes** in the **Options** menu first (see the “AIDA Image Analyzer Basic Concepts and Features User's Manual”). If these settings are not correct further image evaluation will not result in meaningful results.

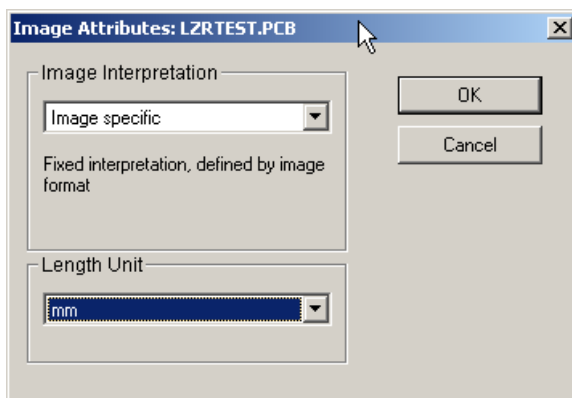


Figure 3-1 Setting Image Attributes

To enable signal intensity evaluation the regions of interest must be marked by an enclosure of the appropriate shape. In the beginning you might find it difficult to exactly encircle a region of interest. Some experience is needed to get this done on the first attempt.

To select regions of interest:

- 1 Click the appropriate button (here circle) in the **Region Creation** pane of the **Region Determination** toolbox to select a shape (alternatively click the circle symbol on the toolbar).



Figure 3-2 Selecting Circle Tool

- 2 Position the mouse pointer over the top left edge of the region in the image, press the mouse button and drag the mouse to the lower right edge of the region to be assigned.

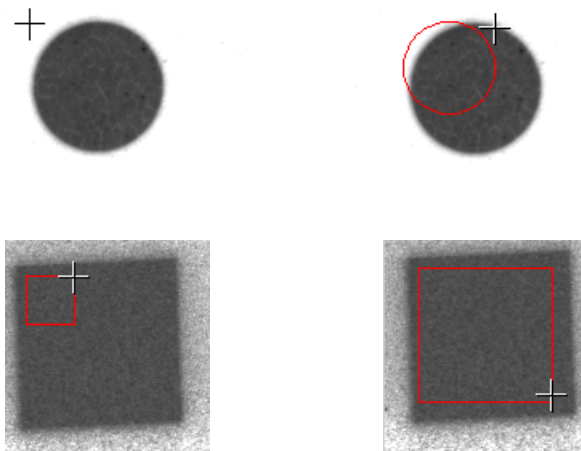


Figure 3-3 Marking Region

- 3 Release the mouse button.



- 4 Drag on the handles of the overlay to adjust the shape of the region overlay.

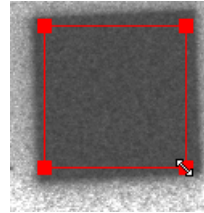
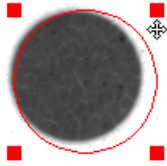


Figure 3-4 *Adjusting Region Shape*

- 5 Click in the middle of the region to adjust the position of the entire region.

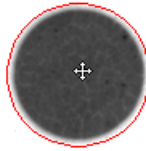


Figure 3-5 *Adjusting Region Overlay Position*

To rotate a region overlay:

- 1 Click the left mouse button to switch to rotation mode.

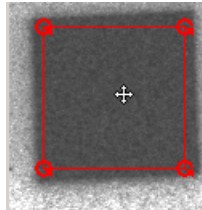


Figure 3-6 *Activating Rotation Mode*

- 2 Drag on a handle to rotate the overlay.

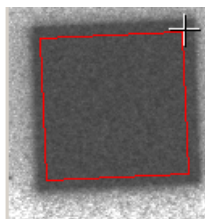


Figure 3-7 Rotating an Overlay

- 3 Click the left mouse button again to go back to the relocation mode.
You might need multiple rounds of adjusting in the beginning.
- 4 After the region is correctly assigned, click the right mouse button or press the ENTER key to save it.

The same strategy is followed for the squares, circles, and ellipses. The ellipses tend to be the most difficult to align.

The **Polygon** tool and the **Freehand Drawing** tool  work similar as for known graphic programs:



Using the **Polygon** tool, every edge of the new region is clicked, whereas using the **Freehand Drawing** tool the mouse button is hold during the manual encircling.

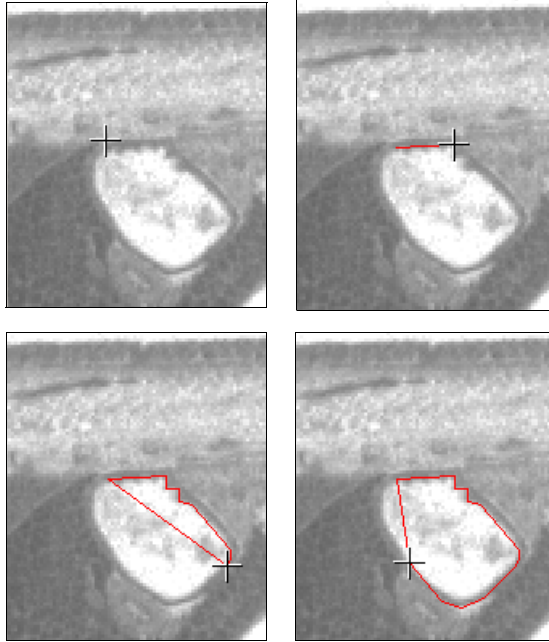


Figure 3-8 Using the Polygon Tool

To edit these polygons a third mode exists besides the relocation and turning mode. In this third mode all handles (edges) of the region will be displayed and their position can be changed. This allows you to reshape the region.

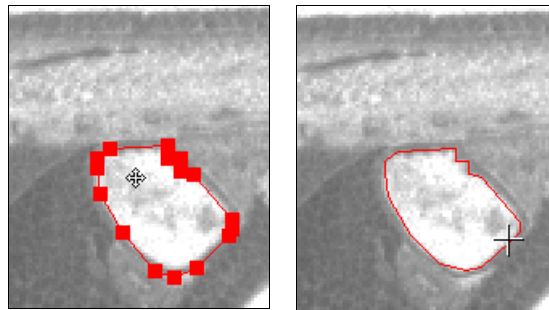


Figure 3-9 Editing Polygon Overlays

Finally the **AutoContour** tool will connect pixels of similar intensity to encircle an object automatically.

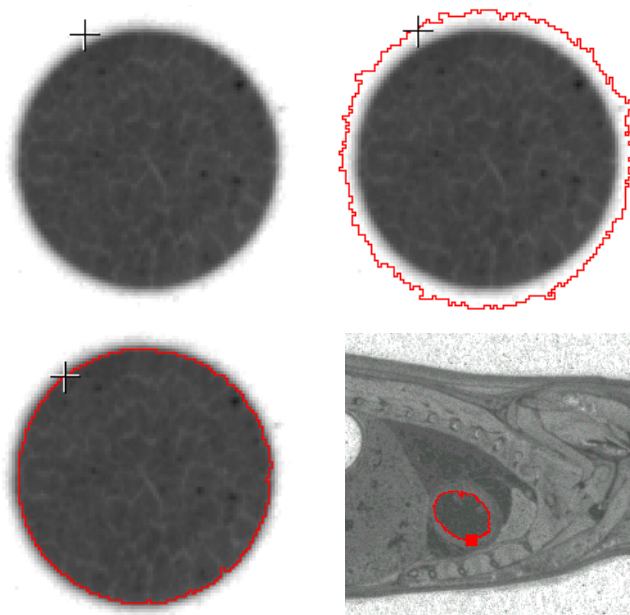


Figure 3-10 Using the AutoContour Tool

To mark a region using the AutoContour tool:

- 1 Select the **AutoContour** tool.
- 2 Point to a position within the border area of an object that you would like to encircle (example above: heart of a rat cut autoradiography), press the mouse button and shift around carefully within the object until the correct border is selected.
- 3 Release the mouse button.

On release, the border is marked and can be edited as usual.

You can cancel any overlay (region) editing action anytime you want by simply pressing the ESC key.

The numbering of the regions can be configured using the **Region Label and Name** controls of the **Region Determination** toolbox (for details, refer to the Region Label and Name pane in the “Region Determination Toolbox” chapter of this manual). Adjust the settings to your experimental design and personal preferences.



Using Arrays (Matrices)

It can be helpful to use matrices to evaluate regular patterns of regularly shaped regions (e.g. slot or dot blots, multiwell plates, etc.).

To create a matrix:

- 1 Mark the first region of the regular pattern in question using the appropriate controls of the **Region Creation** pane on the **Region Determination** toolbox.
- 2 Accomplish the region creation by clicking the right mouse button or alternatively pressing the ENTER key.
- 3 Click the **Create Matrix** button on the **Region Creation** pane, to specify the appropriate matrix layout for the region pattern.

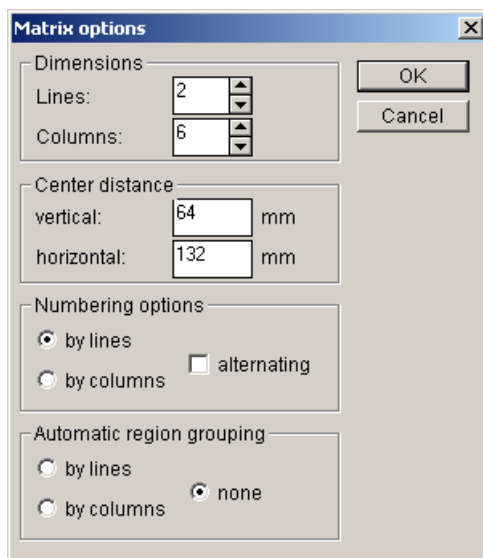


Figure 3-11 Matrix Options Dialog Box

- 4 Enter the number of lines (2 in this examples) and columns (6 in this examples) as well as the distances (x and y- direction) between the center of the regions.

This will be easy for scanners (just measure the original and enter the actual distance) but difficult for camera images (trial and error). However, the work is worthwhile, because all regions will be drawn and assigned at the same time and the same array can be used for another image, even if the size of the image is somewhat different from the original one.



Figure 3-12 Matrix Creation

To save the matrix:

- 1 Choose **Template** from the **Export** submenu of the **File** menu.
- 2 Type a name for your matrix template in the text box of the dialog box that appears (e.g. slotblot.atf).

You can apply your saved template to a similar image from the same kind of application by selecting the **Template** command from the **Import** submenu and then choosing the appropriate template from the displayed file list in the dialog box that appears. The matrix region will be displayed in one single frame which can be moved and reshaped as if it would be only one region.

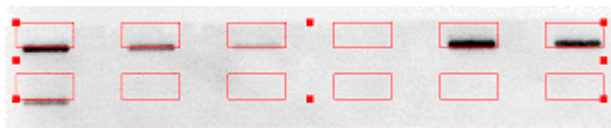


Figure 3-13 Matrix Creation Completed

You can relocate the matrix template by dragging the frame in the appropriate direction.

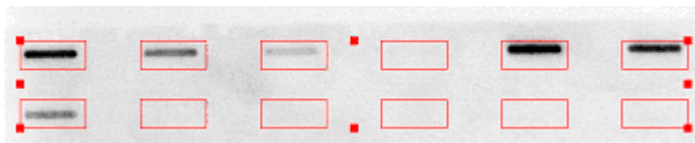


Figure 3-14 Relocating Matrix Template



To rotate the template, just double-click it and drag one of the handles that appear.



Figure 3-15 Rotating Matrix Template

Finally accomplish your adjustments by right-clicking the matrix or alternatively pressing ENTER.

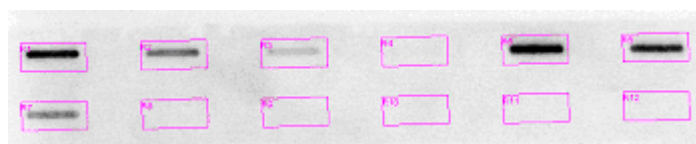


Figure 3-16 Matrix Template Adjustments Completed

Defining a Background Signal

It is advisable to determine the background signal before quantifying the signals.

Since in many applications the background can vary considerably from location to location, the possibility to assign an individual local background around a region can help to evaluate the signals. Note, that local backgrounds can be defined for *rectangular* and *ellipsoidal* regions only. It should also be noted that the background around the region must be clearly separated from the signal. If e.g. a 2D gel spot is assigned, it is likely that the outer borders of the signal come to lie under the background area, because they are very difficult to see for the human eye.

To define a local background region (rectangular and ellipsoidal regions only):

- 1 Mark the respective image areas using the **Region Creation** tools of the **Region Determination** toolbox.
- 2 To activate the local background function, click the **Local Bkg** button on the **Integration** pane of the **Region Determination** toolbox.

This opens the **Local Bkg Settings** dialog box.

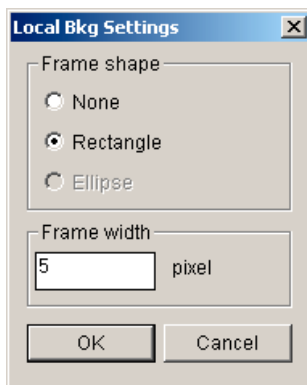


Figure 3-17 Local Bkg Settings Dialog Box

- 3 Enter the distance (in this example in pixels) around the assigned region.



The resulting regions around the assigned areas will be used to determine the background signal and subtract it from the integration results.

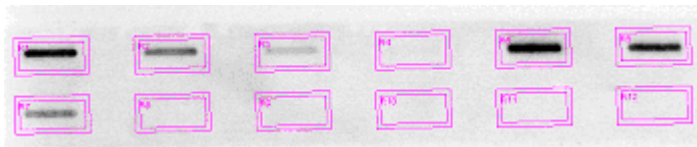


Figure 3-18 Regions with Marked Local Background Areas

To define a background for all other region shapes:

- 1 Select more than one region and group them using the contextual menu of the **Region Report** window.
- 2 Select one of the member of the region group and select the **Background** option on the Integration pane of **Region Determination** toolbox.

If a region group does not contain any background region the background regions of group 0 are applied for the region by default.

Performing Calibrations

The signal intensity of a region can be compared with the signal intensity of other regions (relative comparison) or normalized by standards in the same image, or even in other samples of the same series.

For the normalization (calibration), it is necessary to define reference regions for the creation of standard curves.

To assign standards:

- 1 Select the **Manual Assignment** tool from the **Region Calibration** toolbox.
- 2 Click on a region.



The **Region Calibration** dialog box pops up and prompts you to enter the standard value und unit of measurement.

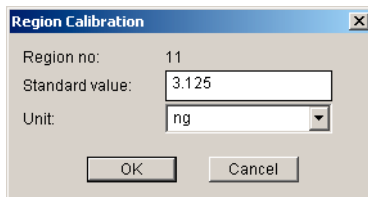


Figure 3-19 Entering Standard Values and Unit of Measurement

In the example shown below the regions R6 to R11 are a two-fold dilution series of a standard. Region R5 was assigned as a background region. Region R1 to R4 are various dilutions of a sample that should be analyzed.

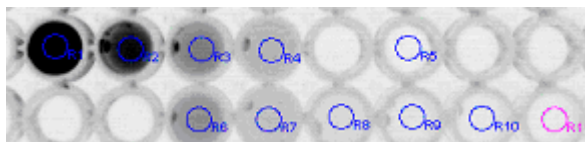


Figure 3-20 Region Calibration Example

- 3 Enter the appropriate standard value und click **OK** to assign it to the region.



In the **Region Calibration** toolbox the entered standard values are listed below the **Standard** drop-down menu.

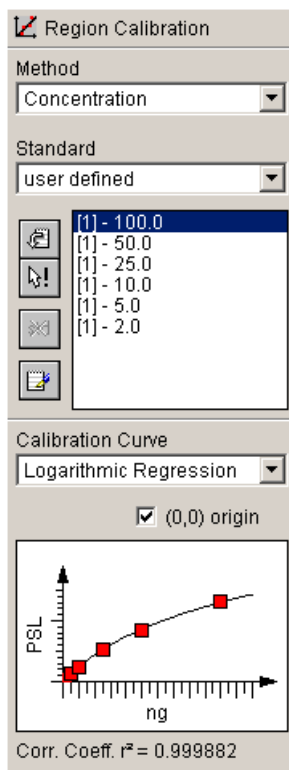


Figure 3-21 Region Calibration Toolbox With Entered Standard Values Displayed

The list of standard values can be saved for later use as a standard list. Standard values from saved lists can be used to assign all standards by merely clicking at each region subsequently.

- 4 Select a **Calibration Curve** type from the **Calibration Curve** drop-down menu.

The type of the calibration curve can be selected on the basis of theoretical considerations or simply to fit the observed situation. The resulting calibration curve can be immediately evaluated and the r^2 value will give a parameter to judge the fit (should be next to 1).

- 5 Turn on the **(0,0) Origin** option if useful.

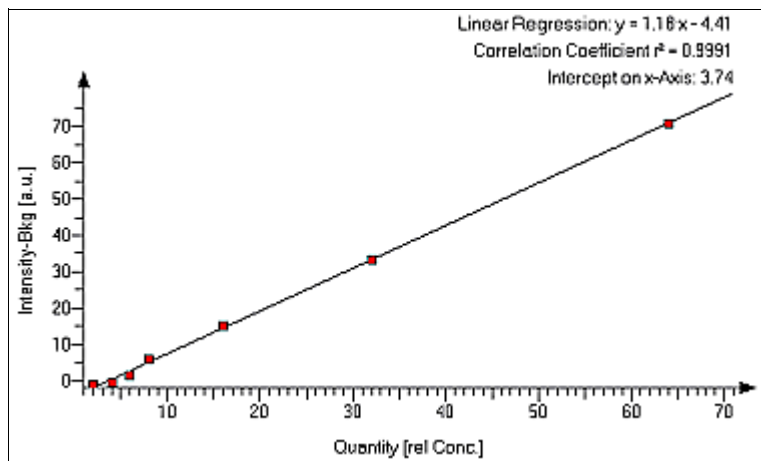


Figure 3-22 Calibration Graph Example

As mentioned above, a correlation coefficient value of 1 identifies a perfect correlation, a value of 0 indicates full independence of values. Good standard curves should have value very near to 1.



Using Nametables

Nametables are used to name regions for reference purpose.

Creating and Editing Work Nametables

Work nametables are used to assign names to regions. Any user can create a new or edit an existing work nametable.

To create a new work nametable:

- 1 On the **Region Determination** toolbox click the **Select Name List** button.

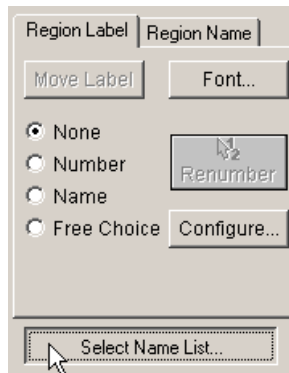


Figure 3-23 Region Determination Toolbox: Select Name List Button

The **Region Nametables** dialog box appears showing the current work nametable's list of names and a dropdown list with the names of all existing work nametables.

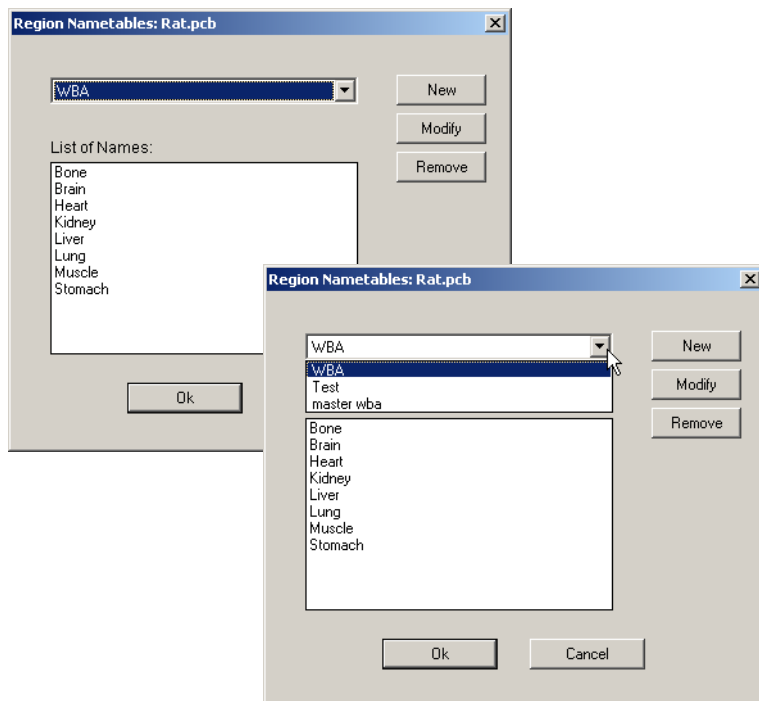


Figure 3-24 Region Nametables Dialog Box

2 Click **New**.

The **Nametable Editor** dialog box appears.



- 3 Type a name for the work nametable in the **Title** text box and a list of names in the **List of Names** text box.

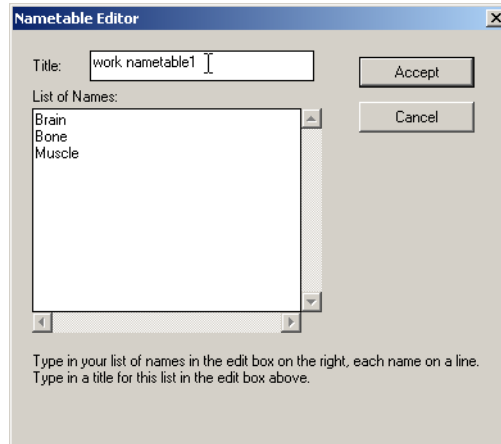


Figure 3-25 Nametable Editor Dialog Box

- 4 Click **Accept** to confirm the changes.

To modify a work nametable:

- 1 On the **Region Determination** toolbox click the **Select Name List** button.
The **Region Nametables** dialog box appears.
- 2 Click **Modify**.
The **Nametable Editor** dialog box appears.
- 3 Edit the entries in the in the **List of Names** list box.
- 4 Click **Accept** to confirm the changes.

Assigning Names to Regions

To assign names to regions:

- 1 Select a region.

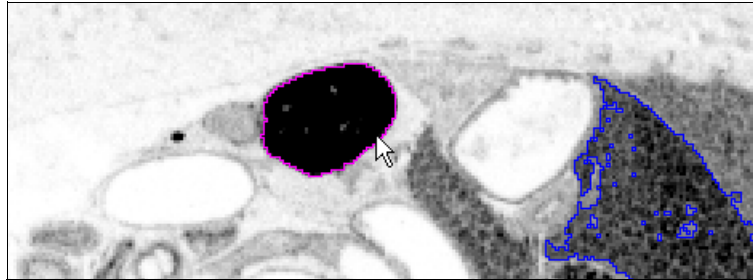


Figure 3-26 Selecting a Region

- 2 On the **Region Determination** toolbox, select the **Region Name** tab.

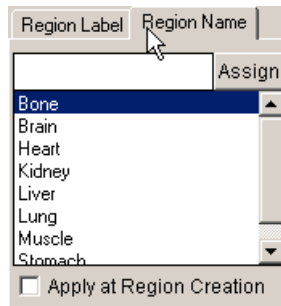


Figure 3-27 Selecting Region Name Tab



- 3 Select the appropriate entry from the list to assign the name to the selected region.
The list shows the names defined in the current work nametable.

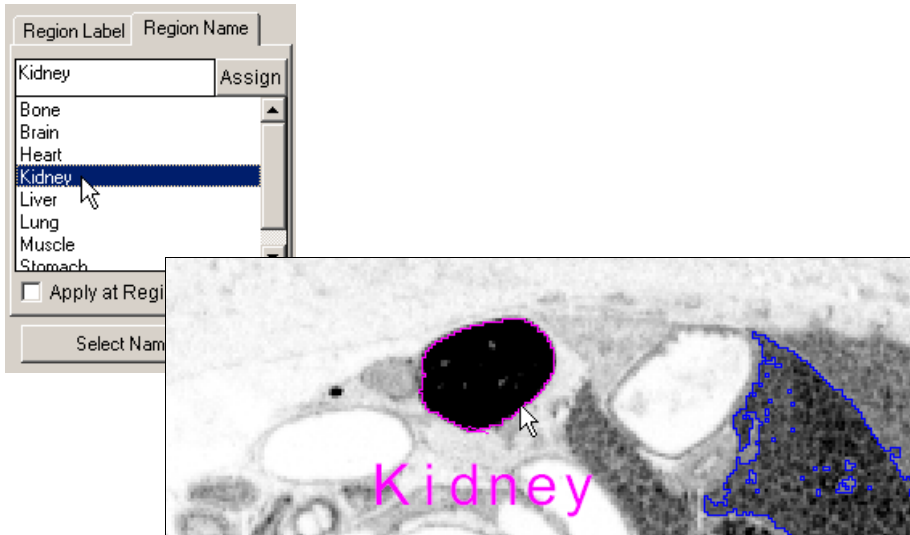


Figure 3-28 Selecting a Name

To change a region name:

- 1 Select the region whose name you want to change.

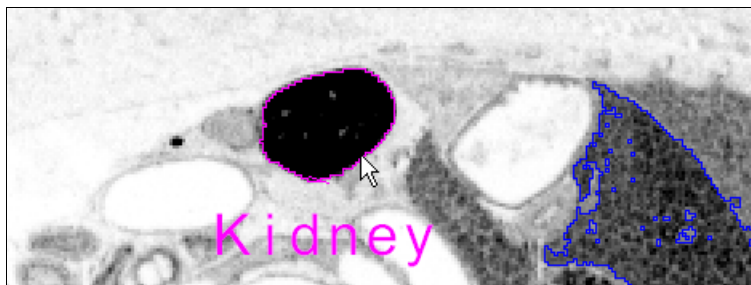


Figure 3-29 Selecting the Region



- 2 Click the appropriate name in the **Region Name** list.



Figure 3-30 Changing Name

To assign names automatically upon creation of a region:

- 1 On the **Region Determination** toolbox, select the **Region Name** tab.

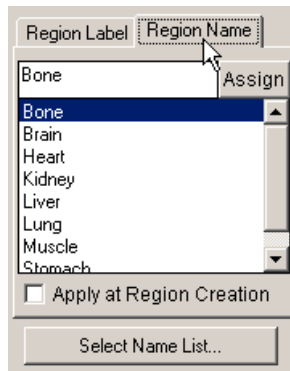


Figure 3-31 Selecting Region Name Tab



2 Click **Apply at Region Creation**.

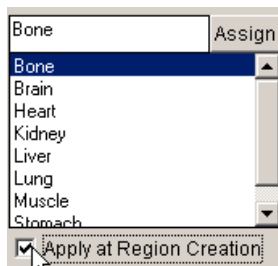


Figure 3-32 Checking Apply at Region Creation

3 Select a tool from the **Region Creation** pane and mark regions successively.



Figure 3-33 Selecting Region Creation Tool

The names in the **Region Name** list are assigned one after another.

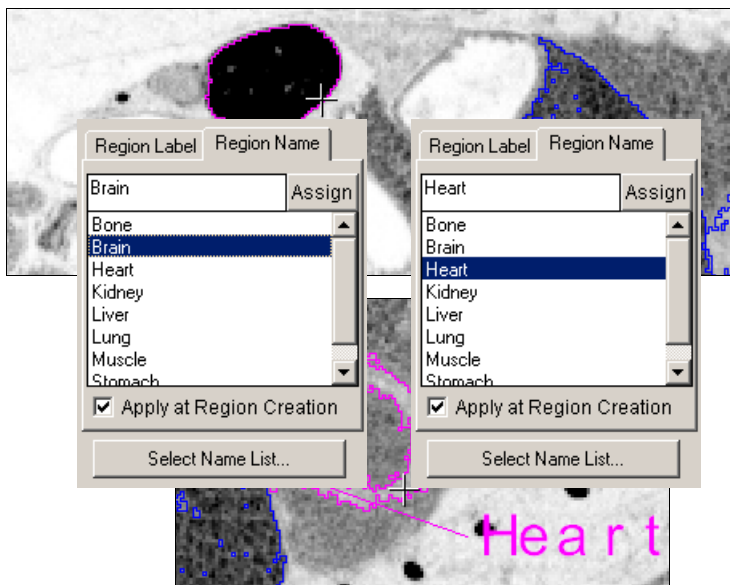


Figure 3-34 Assigning Names successively

